Effects of natural iron fertilisation by baleen whales and Antarctic krill on the Southern Ocean carbon cycle

By

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Submitted in fulfilment of the requirements for the Degree of

Doctor of Philosophy

University of Tasmania

November 2016

Abstract

Primary productivity in large areas of the Southern Ocean, known as High Nutrient Low Chlorophyll (HNLC) regions, is limited by the availability of a key micronutrient – the trace element iron (Fe). Iron is required for biochemical processes such as photosynthesis and respiration, as well as in the reduction of inorganic nitrogen species. There is growing evidence that marine animals could play an important role in recycling Fe through their diet and subsequent defecation, reviewed in Chapter 1. This dissertation adds significantly to our understanding on the influence of Antarctic krill (*Euphausia superba*) and baleen whales on the biogeochemical cycling of Fe, and other biologically important trace elements, in the Southern Ocean.

The concentration of Fe, and other biologically important trace elements such as manganese (Mn), which is essential for carbon fixation; zinc (Zn), cadmium (Cd), and cobalt (Co) for CO_2 acquisition; Zn and Cd for silica uptake by large diatoms; Co and Zn as calcifiers; copper (Cu) and Fe for nitrification, denitrification and organic N utilization; Zn for organic phosphorus (P) utilization; and Cu for methane oxidation, in whole Antarctic krill and baleen whale muscle and faecal samples were measured in Chapter 2 to explore the biogeochemical role of Antarctic krill and baleen whales in the Southern Ocean. Antarctic krill were found to be rich in Fe (174.5 ± 0.5 mg kg^{-1}), and these other biologically important trace elements. The elements stored in Antarctic krill are then transferred into the whales as they are consumed. Adult whales build blubber (fat) instead of muscle during their feeding season in Antarctic waters; consequently much of the nutrients get defecated. Iron concentrations in whale faecal material were found to be 145.9 ± 135.4 mg kg^{-1}, over 10 million times higher than background seawater concentrations. Similarly, concentrations of other biologically important trace elements were elevated in whale faecal material compared to Southern Ocean seawater. The trace element to carbon ratio further suggests that whales are concentrating carbon and actively defecating trace elements.

Based on these high Fe concentrations, a preliminary model was built in Chapter 3 to examine the potential Fe fertilisation by blue, fin and humpback whales, and Antarctic krill on the phytoplankton growth in the Southern Ocean. The model was used to examine the influence of

historical whaling practices on the efficiency of Fe recycling. A local sensitivity analysis, which allowed for the use of the range of values (minimum, mean and maximum) for each parameter was used. The model suggested that historical populations of blue, fin and humpback whales might have enhanced primary productivity in the Southern Ocean. However there is a high degree of uncertainty around the magnitude of this enhancement due to uncertainty in model parameter estimates, which prioritised key areas for future research. Based on the model, the most influential parameters were: the Fe concentration in krill, the carbon-to-iron uptake ratio by phytoplankton, persistence and bioavailability of whale faecal material, and the consumption rate by whales.

In order to constrain the key parameter "Fe concentration in krill", the concentration of Fe in whole krill, distinct krill tissue material and krill faeces was analysed in Chapter 4. The results demonstrate that much of the Fe in krill is stored in the stomach (6 – 98 mg kg^{-1}) and digestive gland (14 – 82 mg kg^{-1}), and excreted as faecal material (683 – 1,039 mg kg^{-1}), instead of being stored in the muscle (4 – 7 mg kg^{-1}). This implies that Antarctic krill are ingesting more Fe than they require for physiological processes, and may be important recyclers of Fe in the Southern Ocean. Calculations suggest that the high Fe concentrations in the stomach and digestive gland can influence the overall Fe concentration in Antarctic krill. The large variability reported in the existing literature is very likely the result of a combination of seasonal and regional difference in quality and the quantity of their diet.

The "persistence and bioavailability of whale faecal material" in surface seawater was subsequently investigated in Chapter 5 by size fractionating Fe particles in whale faecal samples into 4 different size fractions (<0.2 μm, 0.2 – 10 μm, 10 – 60 μm and >60 μm), and measuring the leaching Fe particles over time. The results suggest that the total particulate fraction (>0.2 μm, 5,026 – 22,526 nmol L^{-1}) dominated the total Fe pool (5,780 – 23,053 nmol L^{-1}). The concentrations of dissolved Fe (186 – 754 nmol L^{-1}) and particulate Fe in whale faecal samples, however, were significantly higher than published Southern Ocean surface seawater concentrations, and most other Fe sources in the region. A range of processes such as remineralisation, leaching, aggregation, precipitation, the recycling of biogenic particulate Fe in surface seawater, and leaching of particulate Fe will influence the bioavailable pool of Fe.

Between 1 and 7% of the Fe leached from whale faeces in the first 5 minutes. Although the solubility of faecal particles seems low, the concentration of Fe being leached is high (51 - 143 nmol L^{-1}), and is greater than the solubility of Fe in seawater. In addition, calculations on the sinking rate of these particles (60 μm, 10 μm and 0.2 μm would sink at a rate of 3 m day^{-1}, 0.08 m day^{-1} and 3.3 x 10^{-5} m day^{-1} respectively) suggest that they may remain in the water column for an extended period, however many of these particles may aggregate and precipitate, or be transported laterally.

In summary, this dissertation has demonstrated that Antarctic krill acts as an efficient reservoir of Fe, with much of the consumed Fe being stored in the digestive organs and not incorporated into the muscle. Baleen whales then recycle the Fe stored in Antarctic krill through their diet and subsequent defecation. Although whale faecal material consists mostly of particulate Fe, the concentration of dissolved Fe in whale faecal material is comparable to marine ice and continental ice, but considerably higher than other sources in the region. This suggests that baleen whales could play an important role in recycling Fe to HNLC regions of the Southern Ocean. Future research should focus on examining the importance of organic ligands in whale faecal material, the response of phytoplankton to faecal Fe, and the influence of historical whaling processes on the efficiency of Fe recycling in the Southern Ocean.

Declaration of originality

This thesis contains no material which has been accepted for a degree or diploma by the University or any other institution, except by way of background information and duly acknowledged in the thesis, and to the best of my knowledge and belief no material previously published or written by another person except where due acknowledgement is made in the text of the thesis, nor does the thesis contain any material that infringes copyright.

Lavenia Ratnarajah 30 November 2016

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Lavenia Ratnarajah 30 November 2016

Acknowledgements

I would like to thank my 4 supervisors, A/Prof Andrew Bowie, Dr Delphine Lannuzel, Prof Steve Nicol and Dr Klaus Meiners for their advice and encouragement through my dissertation research. The unwavering support that I have received from the 4 of you has made me a better scientist, and a better person. You truly were the dream team of supervisors!

Throughout my PhD I have also received immense support from Dr Pier van der Merwe, A/Prof Ashley Townsend, Dr So Kawaguchi, Dr Jess Melbourne-Thomas, Dr Martin Marzloff, Dr Kathrin Wuttig, Dr Melanie East, A/Prof Ari Friedlaender, Dr Ingrid Obernosterer, Prof Stéphane Blain, Prof Tom Trull, Rob King, Dr Elanor Bell, Dr Virginia Andrews-Goff and Dr Mike Double. This thesis covers a broad knowledge field, and I would like to thank all of you for invaluable mentoring throughout my candidature. I would also like to specially thank Prof Tony Worby, and Wenneke ten Hout for their support and ensuring that all ACE CRC PhD students were able to work in an inviting and comfortable environment.

Thank you to all the staff and students of IMAS, ACE CRC and AAD who have also been incredibly supportive, and creating a fun working environment. A special thanks goes out to Thomas Holmes, Christine Weldrick, Delphi Ward, Clara Peron, Clemence Benoit, Matthieu Bressac, Alvaro Gomez, Sara Padilla, Fanny Noisette, Daniela Farias, Julie Janssen, Rene Reinfrank, Scott Ling, Jess Erickson and Andrew Jackson for your constant encouragement throughout the tough times, and the drinks that flowed during the good times. Thank you Jemina Stuart-Smith for being the only person to read through my Honours thesis and my PhD thesis! Thank you to my family for always being there, and to Tino for always making me happy. Thank you to the staff at Castray Esplanade café for caffeinating me throughout my PhD. Lastly, I would like to thank the Holsworth Wildlife Research Endowment for funding my dissertation research, and the Antarctic Climate and Ecosystems Cooperative Research Centre for the top-up scholarship.

Statement of co-author contributions

The following collaborators and institutions contributed to the publication of the work undertaken as part of this thesis:

1. **Ratnarajah L,** Bowie AR, Lannuzel D, Meiners KM, Nicol S (2014) The Biogeochemical Role of Baleen Whales and Krill in Southern Ocean Nutrient Cycling. PLoS ONE 9(12): e114067. doi:10.1371/journal.pone.0114067

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LR, ARB, DL, KMM and SN designed the project. DL and KMM performed laboratory analysis. LR took the lead role in data analysis, manuscript preparation and reviews, with ARB, DL, KMM and SN assisting in manuscript drafting.

2. **Ratnarajah L,** Melbourne-Thomas J, Marzloff MP, Lannuzel D, Meiners KM, Chever F, Nicol S, Bowie AR. (2016) A preliminary model of iron fertilisation by baleen whales and Antarctic krill in the Southern Ocean: sensitivity of primary productivity estimates to parameter uncertainty. Ecological Modelling 320: 203 – 212 doi:10.1016/j.ecolmodel.2015.10.007

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3. **Ratnarajah L,** Nicol S, Kawaguchi S, Townsend AT, Lannuzel D, Meiners KM, Bowie AR. (2016) Understanding the variability in the iron concentration of Antarctic krill. Limnology and Oceanography 61:1651 – 1660 doi: 10.1002/lno.10322

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4. **Ratnarajah L,** Lannuzel D, Townsend AT, Meiners KM, Nicol S, Friedlaender AS, Bowie AR. (in revision) Physical speciation and solubility of iron from baleen whale faecal material. Marine Chemistry

Ratnarajah L 70%, *Lannuzel D 5%, Townsend AT 5%, Meiners KM 5%, Nicol S 5%, Friedlaender AS 5%, Bowie AR 5%.*

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5. **Ratnarajah L,** Bowie AR. (2016) Nutrient Cycling: Are Krill a Previously Overlooked Source in the Marine Iron Cycle. Current Biology http://dx.doi.org/10.1016/j.cub.2016.08.044

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LR and ARB co-wrote the dispatch article reviewing the current literature on Antarctic krill in relation to the marine iron cycle. This is discussed in the summary presented in Chapter 6.

We the undersign agree with the above stated proportion of work undertaken for each of the above published and in review peer-reviewed manuscripts contributing to this thesis:

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Table of Contents

List of Figures

List of Tables

1. Introduction

1.1 Role of iron in regulating the Earth's climate

The Earth's climate is sensitive to atmospheric carbon dioxide (CO_2) levels. In turn, the concentration of atmospheric CO_2 is greatly influenced by the growth of phytoplankton in the Southern Ocean; with the region estimated to be responsible for approximately 30% of the global carbon uptake (Schlitzer 2002). The growth of phytoplankton in large areas of the Southern Ocean, however, is limited by the availability of a key micronutrient – iron (Fe) (Martin and Fitzwater 1988, Martin 1990, Martin et al. 1990, Boyd et al. 2000, Blanchot et al. 2001, Tsuda 2003, Tyrrell et al. 2005, Blain et al. 2007). Iron is required by phytoplankton for nitrogen acquisition and assimilation, detoxification of reactive oxygen species, deoxyribonucleotide synthesis, chlorophyll synthesis, and electron transfer in respiration and photosynthesis (Morel et al. 2003). Despite the extensive requirement of Fe for metabolic processes, Fe uptake by phytoplankton in large areas of the Southern Ocean is constrained by the limited supply, and its physico-chemical properties in the water column. Elucidating the mechanisms that control Fe supply into the Southern Ocean is central to understanding the mechanics of ecosystem functioning both locally and over broad spatial scales.

1.2 Iron in the Southern Ocean

The deposition of atmospheric dust from the surrounding continents is considered to be the predominant supply of Fe into the ocean (Boyd et al. 2004, Jickells et al. 2005, Cassar et al. 2007). However, the relative importance of atmospheric Fe deposition is highly variable depending on oceanic regions. For instance, atmospheric Fe from the Sahara desert is a major source of Fe into the eastern Atlantic Ocean and Mediterranean Sea (Guieu et al. 2002, Sarthou et al. 2003). In contrast, because Antarctica is ice-covered, and circumpolar winds and currents isolate much of the Southern Ocean from the other continents, the input of atmospheric Fe into the Southern Ocean is relatively lower compared to other Southern Ocean Fe sources. In the Southern Ocean and Antarctic region, the melting of sea ice (Sedwick and Di Tullio 1997, Lannuzel et al. 2007), ice shelves (Herraiz-Borreguero et al. 2016a) and icebergs (Smith et al. 2007, Lin et al. 2011, Duprat et al. 2016), hydrothermal vents (Tagliabue et al. 2010, Klunder et al. 2011), upwelling (de Baar et al. 1995), and the weathering of shelf sediments (Sedwick et al.

2008, Bowie et al. 2009) also contribute to the supply of Fe to varying degrees of spatial and temporal importance, which is then mediated by local mixing and global circulation. Areas remote from these sources remain biologically unproductive throughout the year.

Uptake of Fe by phytoplankton is further influenced by the size of the Fe particle, the favourable complexation of certain forms of Fe by organic ligands, competition by bacteria, and the oxidation state. The size fractination between "dissolved" and "particulate" (operational cut-off of 0.2 or 0.4 μm) phases is the most commonly used as an approximation of element bioavailability, with dissolved Fe being considered as the most accessible form for biological uptake despite the particulate Fe fraction being the dominant pool of total Fe in the water column (de Baar and de Jong 2001). The dissolved Fe pool can be further partitioned into two smaller fractions, soluble Fe (<0.02 μm) and colloidal Fe (between 0.02 μm to 0.2 or 0.4 μm). The relative importance of these fractions is uncertain. Wu et al. (2001) demonstrated that the soluble fraction may be more bioavailable than the more chemically dynamic colloidal fraction, while Honeyman and Santschi (1989) demonstrated that the colloidal fraction could aggregate into larger particles and settle from the water column. In contrast, Hassler et al. (2011a) demonstrated the role of organic ligands in enhancing the colloidal fraction within the water column.

There is strong evidence that 99% of dissolved Fe in the Southern Ocean is complexed by organic ligands, making them more bioavailable (Boye et al. 2001, Croot et al. 2004, Hassler and Schoemann 2009, Boye et al. 2010, Ibisanmi et al. 2011, Thuróczy et al. 2011). Organic ligands include siderophores (Vraspir and Butler 2009) – a compound produced by bacteria; poryphyrins (Hassler and Schoemann 2009) – biologically produced compounds, which include chlorophylls and chlorophyll breakdown products such as phaeophytin, hemes and vitamin B12; and saccharides (Hassler et al. 2011a, Hassler et al. 2011b), which are complex molecules that are challenging to chemically characterise.

Heterotrophic bacteria can constitute up to 50% of the total particulate organic carbon in open ocean waters. In the waters around Kerguelen Islands, heterotrophic bacteria were found to be co-limited by both Fe and C (Fourquez et al. 2015, Obernosterer et al. 2015). The phytoplankton-bacteria relationship is highly complex. Phytoplankton are a source of C for heterotrophic

bacteria, yet phytoplankton and bacteria compete for the bioavailable Fe fraction. In addition, bacteria produce strong organic ligands (e.g. enterobactin, a strong siderophore, which is produced by bacteria such as *Escherichia coli*) that make Fe more bioavailable for uptake by phytoplankton. Consequently, the availability of Fe for uptake by phytoplankton is further constrained by the multifaceted connection with bacteria in the water column.

In terms of chemical forms, Fe^{2+} is considered to be the more bioavailable fraction, due to the low solubility of the thermodynamically stable Fe^{3+} redox species. In oxic waters, Fe^{2+} is rapidly oxidised into Fe^{3+} by oxygen and hydrogen peroxide. However, Fe^{3+} can be reduced to Fe^{2+} through photochemical reduction of colloidal Fe (Wells and Mayer 1991, Rijkenberg et al. 2005) or reduction of organically bound Fe^{3+} (Barbeau 2006, Rijkenberg et al. 2006). Although the reoxidation of Fe^{2+} occurs quite rapidly (1 hour) (Millero et al. 1987), the presence of organic ligands (Gledhill and van den Berg 1994, Barbeau 2006, Boye et al. 2010, Hassler et al. 2012), and generally low hydrogen peroxide concentrations in the Southern Ocean (Sarthou et al. 1997) may slow the oxidation rates.

All these factors combined result in the depletion of bioavailable Fe in large areas of the Southern Ocean surface waters (Fe concentration ~ 0.1 – 0.5 nM, Tagliabue et al. 2012). Recently, it has been suggested that marine animals could also play an important role in recycling Fe in the Southern Ocean (Smetacek and Nicol 2005, Nicol et al. 2010). Such recycling is thought to keep the nutrients in suspension and bioavailable for phytoplankton in Southern Ocean surface waters (Smetacek and Nicol 2005, Nicol et al. 2010).

1.3 Role of marine animals in the cycling of iron

Phytoplankton are the foundation of the marine food web. With sufficient sunlight and nutrients, phytoplankton photosynthesize, converting inorganic carbon from surface seawater into particulate organic carbon, on which other components of the ecosystem depend. Herbivorous animals including krill graze on phytoplankton as their primary food source. In turn, larger marine animals such as fish, flying seabirds, penguins, seals, and whales consume these herbivores. Thus, the Fe stored within the cells of phytoplankton are transferred through the various trophic levels of the complex Southern Ocean food web.

My PhD thesis combines experimental research alongside modeling approaches to investigate the biological recycling of Fe by baleen whales and their primary prey, Antarctic krill (*Euphausia superba*) in the Southern Ocean. This research focused on this particular predator-prey relationship because 1) baleen whales feed almost exclusively on Antarctic krill, therefore, it is easier to map the trophic pathway of Fe and 2) whale faecal material is both voluminous and fluid, and is defecated at, or close to the surface (Figure 1.1). This research builds on the background knowledge of the Southern Ocean Fe cycle, and incorporates the marine food web (Figure 1.2).

Figure 1.1 Image of a defecating minke whale in the Southern Ocean around sea ice (size = 7 – 8 m, source J. Brokowski, Australian Antarctic Division 2010)

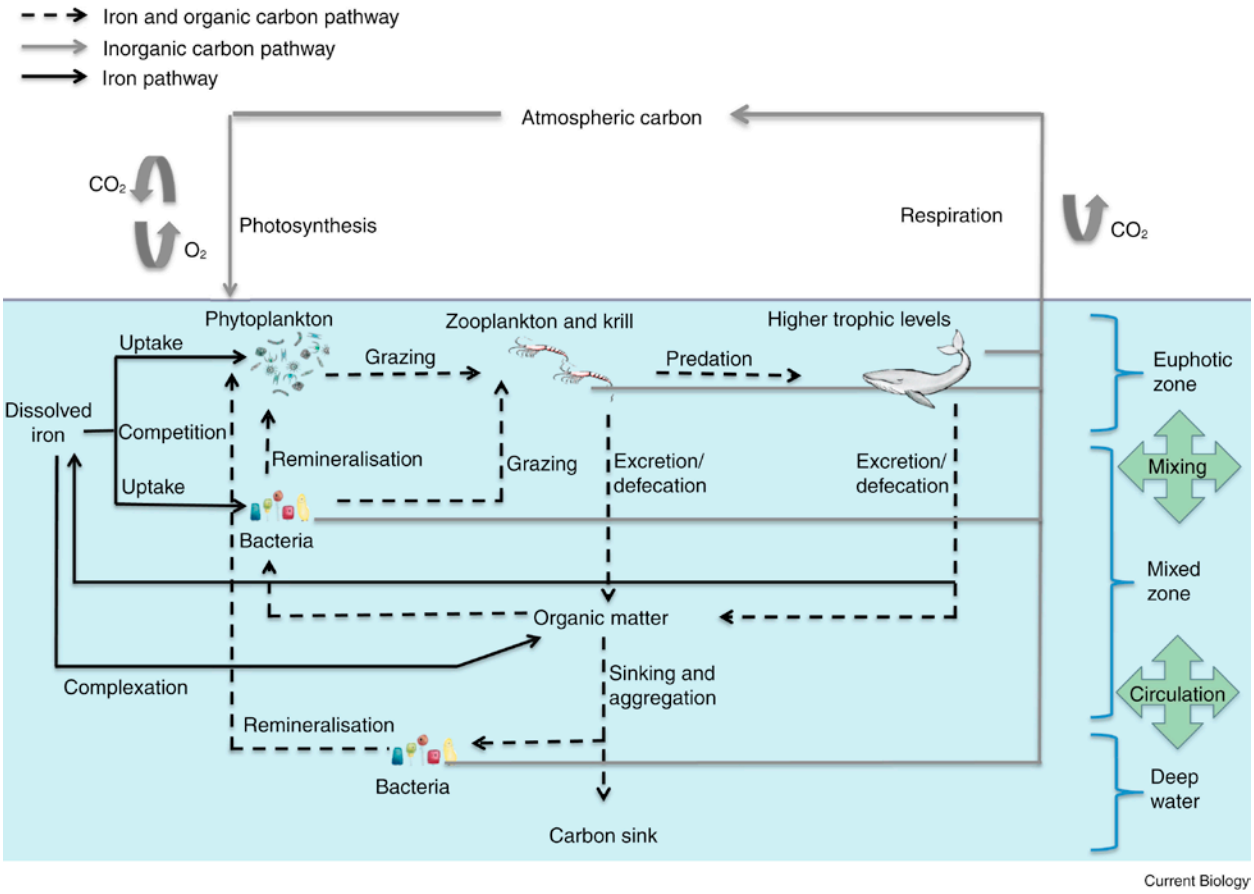


Figure 1.2 Conceptual model on the relationship between iron and carbon cycles in the Southern Ocean (source Ratnarajah and Bowie 2016)

The conceptual model begins with dissolved Fe, derived from the multiple sources mentioned earlier, present in the euphotic zone. From here, there is an initial competition between phytoplankton and the bacterial community for this limiting nutrient (Fourquez et al. 2015, Obernosterer et al. 2015). Despite the interspecies competition, throughout the water column, bacteria are capable of 1) remineralising organic matter and releasing Fe back into the seawater, and 2) producing organic ligands that bind with Fe molecules thus keeping them in solution, for potential uptake by phytoplankton (Hassler et al. 2011a, Hassler et al. 2011b). Scavenging can remove dissolved Fe from the surface seawater when dissolved Fe concentrations are greater than its solubility (Johnson et al. 1997), however complexation with organic ligands protects dissolved Fe from particle scavenging (Street and Payton 2005). This suggests a competitive yet facilitative relationship between phytoplankton and bacteria.

1.3.1 Antarctic krill

Antarctic krill is a keystone species in the Southern Ocean ecosystem. They exhibit a circumpolar distribution around Antarctica (Atkinson et al. 2008) with an estimated biomass of between 100 and 500 million tonnes (Atkinson et al. 2009). Although most of this biomass is located within the upper 150 m of the water column (Demer and Hewitt 1995, Lascara et al. 1999), Antarctic krill have also been shown to actively feed at the seabed (Clarke and Tyler 2008, Schmidt et al. 2011). Being the dominant herbivore in the Southern Ocean, Antarctic krill are able to consume up to 15% of their body carbon day^{-1} (Pakhomov et al. 2002). Thus their almost continuous grazing over spring and summer, both in the surface and at depth, means that they could consume much of the Fe in the annual phytoplankton bloom and act as a biological reservoir of Fe in Southern Ocean surface waters (Nicol et al. 2010), and play an important role in the vertical transfer of Fe (Schmidt et al. 2011). Excess Fe consumed, beyond their metabolic demand, is released back into the seawater, suggesting that they also act as a key recycler of Fe in the Southern Ocean ecosystem (Tovar-Sanchez et al. 2007).

1.3.2 Baleen whales

During their feeding season in the Southern Ocean, baleen whales feed on Antarctic krill as their main dietary source (Lockyer 1981, Nicol et al. 2010). Like all mammals, whales require Fe for the production of red blood cells (haemoglobin), oxygen storage protein in muscles (myoglobin), and Fe containing centres in many enzymes (Ordway and Garry 2004, Ganz and Nemeth 2006). Being a large mammal, their high demand for Fe is met by their high consumption rates over summer. On average, whales are thought to feed in Antarctic waters for at least three to four months (Lockyer 1981). The smaller humpback whale (~13 – 15 m length) is capable of consuming 694 – 874 kg krill day^{-1}. The larger fin whale (~22 – 26 m in length) could consume 1309 – 2258 kg krill day^{-1}, while the largest animal, the blue whale (~25 – 30 m length) is capable of consuming 1682 – 4130 kg krill day^{-1} (Lockyer et al. 1981; Reilly et al 2004).

In addition to size, the metabolic demand for Fe by whales is expected to also vary with life stage (young vs. mature) and sexual maturity (male and female vs. lactating and pregnant females). For instance, young whales would require more Fe to build muscle, so would an injured or pregnant whale. Over summer, adult whales build blubber (fat) instead of muscle to last them through the calving season where food is minimal. Mammals are only able to excrete assimilated Fe by shedding intestinal and skin cells, and through minor blood loss in the intestine (Ganz and Nemeth 2006). Therefore, the Fe absorbed during the growth phase of the whale is retained until adulthood and recycled for future use. Excess Fe, not required by whales is not absorbed through the gut and is recycled into the water column through their faecal material, where it could be an important source of recycled Fe for phytoplankton.

Because whales defecate a liquid slurry at the surface, the recycled Fe may be readily available to phytoplankton. Only one study has measured the concentration of Fe in whale faecal material and found concentrations between 45.7 to 286.5 mg kg^{-1} dry weight, with the smaller whale species, the pygmy blue (*Baleoptera musculus brevicauda*) at the lower end of the range, compared to the larger blue (*Baleoptera musculus*), fin (*Baleoptera physalus*) and humpback (*Megaptera novaeangliae*) whales (Nicol et al. 2010). Natural faecal dilutions with seawater prior to sample collection, however, would heavily influence measured Fe concentrations.

1.4 Aims

The overall aim of this thesis research is to examine the effects of biological recycling of Fe by Antarctic krill and baleen whales on the Southern Ocean carbon cycle. Specifically, this research set out to examine:

I. Whether Antarctic krill and baleen whales are a source of recycled Fe, and other biologically important trace elements, to Southern Ocean surface waters. This information was then compared to background seawater concentrations from various regions in the Southern Ocean to determine their relative importance.

II. The extent of recycling by Antarctic krill and baleen whales in the Southern Ocean, south of 60°. A preliminary model was built using published parameter estimates where available, and assumptions where no information is available. A sensitivity analysis was applied to this model to determine the most influential parameters to model output, which was used to guide future research.

III. The most influential parameter in the preliminary model determined by the sensitivity analysis was the large variability in Antarctic krill Fe concentrations. The Fe concentration in whole Antarctic krill specimens, and in each body part and faecal material was measured to determine if the large variability of Fe concentrations in Antarctic krill observed in the literature is common throughout the Southern Ocean. These results were then compared to other published studies on Fe concentrations in Antarctic krill to investigate the main driver of the Fe concentration in Antarctic krill.

IV. The third and fourth most influential parameter in the preliminary model determined by the sensitivity analysis was the bioavailability and persistence of faecal Fe. The dissolved and particulate Fe fractions were quantified, and the leaching of particulate Fe over time was measured. These results were then compared to other studies to determine the relative importance of recycled Fe.

2 The biogeochemical role of baleen whales and krill in Southern Ocean nutrient cycling

This chapter has been published:

Ratnarajah L, Bowie AR, Lannuzel D, Meiners KM, Nicol S (2014) The Biogeochemical Role of Baleen Whales and Krill in Southern Ocean Nutrient Cycling. PLoS ONE 9(12): e114067. doi:10.1371/journal.pone.0114067

2.1 Abstract

The availability of micronutrients is a key factor that affects primary productivity in High Nutrient Low Chlorophyll (HNLC) regions of the Southern Ocean. Nutrient supply is governed by a range of physical, chemical and biological processes, and there are significant feedbacks within the ecosystem. It has been suggested that baleen whales form a crucial part of biogeochemical cycling processes through the consumption of nutrient-rich krill and subsequent defecation, but data on their contribution are scarce. We analysed the concentration of iron, cadmium, manganese, cobalt, copper, zinc, phosphorus and carbon in baleen whale faeces and muscle, and krill tissue using inductively coupled plasma mass spectrometry. Metal concentrations in krill tissue were between 20 thousand and 4.8 million times higher than typical Southern Ocean HNLC seawater concentrations, while whale faecal matter was between 276 thousand and 10 million times higher. These findings suggest that krill act as a mechanism for concentrating and retaining elements in the surface layer, which are subsequently released back into the ocean, once eaten by whales, through defecation. Trace metal to carbon ratios were also higher in whale faeces compared to whale muscle indicating that whales are concentrating carbon and actively defecating trace elements. Consequently, recovery of the great whales may facilitate the recycling of nutrients via defecation, which may affect productivity in HNLC areas.

2.2 Introduction

Large regions of the Southern Ocean are characterized by low phytoplankton biomass despite high concentrations of major nutrients (e.g. nitrate, phosphate and silicate), and have been characterised as High Nutrient Low Chlorophyll (HNLC) waters (Moore and Abbott 2000). Phytoplankton forms the base of the marine food chain, supporting everything from microscopic animals to large marine mammals (Sheldon et al. 1977, Perissinotto 1997, Frederiksen et al. 2006). It also plays an important role in carbon sequestration by converting carbon dioxide (CO_2) to biomass through photosysnthesis, and through sinking, transferring the carbon to the deep ocean and sea floor sediments (Boyd et al. 2000, Blain et al. 2007). Marine ecosystems can either act as a source or sink of atmospheric CO_2 depending on the relative rates of photosynthesis and overall total respiration. One factor responsible for limiting the accumulation of phytoplankton in HNLC waters has been the availability of essential trace elements, particularly iron (Fe), that are required for biochemical processes such as photosynthesis and respiration, as well as in the reduction of inorganic nitrogen species (Morel et al. 1991).

The major sources of trace elements in marine ecosystems are from atmospheric deposition, continental run-off, shelf sediments, hydrothermal vents and ocean crust (SCOR Working Group 2007). However the Southern Ocean is remote from most of these sources; consequently the concentration of trace elements in surface waters is low. Some of the important trace elements underpinning biogeochemical processes are: Fe and manganese (Mn) for carbon fixation; zinc (Zn), cadmium (Cd), and cobalt (Co) for CO_2 acquisition; Zn and Cd for silica uptake by large diatoms; Co and Zn as calcifiers; Fe for nitrogen (N_2) fixation; copper (Cu) and Fe for nitrification, denitrification and organic N utilization; Zn for organic phosphorus (P) utilization; Fe for synthesis of photopigments; and Cu for methane oxidation (Morel et al. 2003, Morel and Price 2003). As Fe, Mn, and Cu have a short residence time, while Cd, Zn and P have an intermediate residence time in oxygenated waters (Orians and Bruland 1986, Landing and Bruland 1987, Chester 1990, Coale et al. 1996), any mechanism that can increase the persistence of trace elements in surface waters should enhance overall marine primary productivity.

Until recently, the primary biogeochemical role of marine animals was considered to be as consumers of carbon, converting it into fast-sinking faecal material or returning it to the atmosphere through respiration (Huntley et al. 1991). However, a number of recent studies instead suggest that marine animals and seabirds are part of a positive feedback loop that retains nutrients in the surface waters, thus enhancing primary productivity and stimulating carbon export (Jennings and Wilson 2009, Lavery et al. 2010, Nicol et al. 2010, Pershing et al. 2010, Wing et al. 2014).

All animals require a range of nutrients that they mostly obtain from their diet. Different marine animal groups have requirements for particular nutrients: e.g. crustaceans require Cu for their respiratory pigment (Spicer and Saborowski 2010), whereas marine mammals require Fe for the oxygen (O_2) storage protein in muscles; myoglobin (Ordway and Garry 2004). Thus animals tend to concentrate the range of nutrients that are important for their metabolic processes. Marine mammals, being air-breathing, spend most of their lives in the surface layer and are thought to defecate exclusively in the euphotic zone (Kooyman et al. 1981). In addition, some animals inhabit or migrate to water deeper than the euphotic zone, where they feed and then return the scavenged nutrients to the surface layer when they defecate (Clarke and Tyler 2008, Roman and McCarthy 2010). Animals such as seabirds and whales are capable of converting the concentrated elements found in solid form in their prey into a liquid form in their faecal material that is released into the euphotic zone (Smetacek and Nicol 2005, Nicol et al. 2010, Wing et al. 2014). This plume of liquid, rich in trace elements, could act as a fertiliser for phytoplankton production (Smith et al. 2013, Wing et al. 2014). Dense aggregations of large animals may also have a significant local effect on mixing of water and nutrients across the thermocline by generating turbulence (Katija 2012).

The objective of our study was to determine the degree to which a variety of trace elements are concentrated in krill tissue, and subsequently taken up into whale muscle or defecated, to evaluate their potential role in recycling nutrients in the Southern Ocean. In addition to Fe, we report the concentrations of carbon and six other biologically important elements (Cd, Mn, Co, Cu, P and Zn) measured in five species of baleen whales and four species of krill, including Antarctic krill (*Euphausia superba*). Iron concentrations and diet analysis on these samples have

been presented and discussed in Nicol et al. (2010) and Jarman et al. (2002), respectively.

2.3 Methods

2.3.1 Sample collection

Whale muscle samples were collected from stranded and dead blue (*Baleoptera musculus*) and fin (*Baleoptera physalus*) whales in South-western Australia. Blue, fin, sperm (*Physeter macrocephalus*), humpback (*Megaptera novaeangliae*) and pygmy blue (*Baleoptera musculus brevicauda*) whale faecal samples were collected opportunistically from a range of locations by trawling 0.5 mm mesh nets over the surface waters following defecation. Four species of krill (*Nyctiphanes australis, Meganyctiphanes norvegica, Euphausia pacifica* and *Euphausia superba*) were collected from various locations worldwide. All sample tissue and faecal matter were stored in individual 50 mL polycarbonate screw cap bottles, preserved in >70% ethanol and frozen at -20°C until analyses.

2.3.2 Analysis of the trace element concentration

Samples were dried at 60°C until constant weight was attained. Subsequently they were crushed using an acid-cleaned pipette tip and shaken vigorously to homogenise the samples. Digestion of 2 – 100 mg subsamples were performed in acid-cleaned 15 mL Teflon perfluoroalkoxy (PFA) vials (Savillex, Minnetonka, MN, USA) by adding 1 mL of concentrated nitric acid and 0.125 mL of hydrogen peroxide (all Ultrapure, Seastar Baseline, Choice Analytical). The samples were then heated at 125°C for 8 hours on Teflon coated digestion hotplate, housed in a bench-top fume hood coupled with HEPA filters to ensure clean input air (Digiprep, France). Identical procedures were applied to blanks (n = 6) and to two certified referenced materials (n = 5) (DORM-3 fish protein; National Research Council, Ottawa, Canada; and NIST 1566a oyster tissue; National Institute of Standards and Technology, Gaithersburg, Maryland, USA). Certified materials, blanks and samples were resuspended in 10 – 100 mL of 10% v:v nitric acid (Ultrapure, Seastar Baseline) and analysed by sector field inductively coupled plasma mass spectrometry (SF-ICP-MS) (Finnigan MAT ELEMENT 1 Bremen Germany), following methods described in Cullen and Sherrell (1999) and Townsend (2000).

2.3.3 Analysis of carbon

All glass- and metal-ware in contact with the carbon samples were pre-combusted at 450°C for 12 hours. Subsamples (2 – 100 mg) of dried faecal matter were placed in 13 mm diameter silver capsules (Sercon, Australia) and carbon content was then determined at the Central Science Laboratory, University of Tasmania, using a Thermo Finnigan EA 1112 Series Flash Elemental Analyser (estimated precision ~1%).

2.4 Results

2.4.1 Element distribution

Results for certified reference materials are presented in Table 2.1 and were found fit for purpose. Mean and standard deviation of C, Fe, Cd, P, Co, Mn, Cu and Zn for five species of whale faeces, two species of whale muscle and four species of krill are summarised in Table 2.2; with published comparative values of dissolved and particulate trace elements in Southern Ocean surface waters in Table 2.3, marine phytoplankton in Table 2.4, and Antarctic krill and marine mammals in Table 2.5. Concentrations of metals varied between the specimens. In krill tissue, the highest concentration was observed for Zn followed by Fe and Cu. In whale muscle, the highest concentration was observed for Fe followed by Zn and Cu. Lastly, in whale faeces, the highest concentration was observed for Zn, followed by Cu and Fe. Consistently, the three elements with the lowest mean concentrations in krill tissue and whale muscle and faeces were Mn followed by Cd and Co. There are some differences in concentration of the various elements between our results and published data (Table 2.5). These differences may be a result of seasonal or regional effects and variability in trace element concentrations in krill and baleen whales, which is a topic for future studies.

Table 2.1 Elemental analysis using sector field inductively coupled plasma mass spectrometry (SF-ICP-MS) for certified referenced material of fish protein (Certified Reference Material number: DORM-3) and oyster tissue (National Institute of Standards and Technology (NIST), Certified Reference Material number 1566a). Averages listed are the mean of 5 replicates. Recovery values indicate the percentage difference between measured and certified values.

	Fe	**Cd**	**P**	**Mn**	**Co**	**Cu**	**Zn**
DORM-3 referenced values (mg kg^{-1})	347.00	0.29	n/a	4.6	n/a	15.5	51.3
Measured average (mg kg^{-1}) (n= 5)	322.09	0.28	24,865.65	2.92	0.23	14.88	69.55
Standard deviation	42.02	0.03	3,281.6	0.39	0.03	1.47	39.06
Recovery (%)	92.82	96.52	n/a	63.4	n/a	95.99	135.58
NIST 1566a certified values (mg kg^{-1})	539.00	4.15	n/a	12.3	0.57	66.3	830
Measured average (mg kg^{-1}) (n= 5)	477.85	4.13	29,853.89	11.46	0.31	62.60	837.11
Standard deviation	11.84	0.04	1,100.99	0.11	0.02	0.70	7.58
Recovery (%)	88.66	99.49	n/a	93.18	53.65	94.41	100.86

n/a – No certified value given

Mean concentrations of trace elements were higher in whale faecal matter compared to whale muscle and krill tissues. When compared to published Southern Ocean seawater concentrations in HNLC waters (Cullen et al. 2003), the metal content of krill tissue was between 22 thousand (for Co) and 4.8 million (for Fe) times higher than surface water concentrations, while whale faecal matter was between 276 thousand (for Co) and 9.2 million times (for Fe) times higher.

Table 2.2 Carbon, phosphate and trace element concentrations (mean ± standard deviation) in Antarctic krill and whales (mg kg^{-1} dry weight).

Species	**Sample type**	**n**	**Fe**	**Cd**	**Co**	**C** ($x 10^4$)	**P** ($x 10^4$)	**Cu**	**Zn**	**Mn**
Pygmy blue, *Baleoptera musculus brevicauda*	Faeces	7	63.34 ± 17	7.1 ± 2.2	0.5 ± 0.2	17.6 ± 2.5	8.7 ± 2.5	312.2 ± 98.6	607.2 ± 66.0	16.2 ± 9.0
Blue, *Baleoptera musculus*	Faeces	15	161.8 ± 106.5	29.7 ± 8.6	1 ± 0.8	18.5 ± 3.2	9.8 ± 1.9	239.5 ± 68.6	460.8 ± 187.2	33.4 ± 10.6
	Muscle	1	58.3 ± 17.5	0.02	0.006 ± 0.005	5.1	0.03 ± 0.007	1.5 ± 0.2	41.6 ± 4.1	0.3
Fin, *Baleoptera physalus*	Faeces	2	237.4 ± 45.3	42.1 ± 13.1	2.1 ± 1.3	22.1 ± 0.7	12.1 ± 0.4	290.7 ± 11.4	407.1 ± 52.8	30.5 ± 6.9
	Muscle	1	215.7 ± 45.8	0.2 ± 0.3	0.07 ± 0.03	52.8	0.6 ± 0.02	9.2 ± 2.7	108.2 ± 29.2	4.5 ± 0.3
Humpback, *Megaptera novaeangliae*	Faeces	2	118.6 ± 30.1	4.2 ± 3.5	0.9 ±0.8	-	2.9 ± 2.1	74.1 ± 5.2	1099.0 ± 553.0	18.2 ± 10.7
Sperm whale, *Physeter macrocephalus*	Faeces	1	756.7	575	2.2	348.2	6.9	1635.4	2663.6	96
Average among whales	Faeces		145.9 ± 135.4	34.7 ± 88.9	0.9 ± 0.87	19.2 ± 4.5	8.9 ± 3.1	292.4 ± 238.1	621.5 ± 432.9	27.7 ± 16.5
	Muscle		136.9 ± 91.6	0.11 ± 0.19	0.04 ± 0.04	51.9 ± 1.2	0.4 ± 0.2	5.3 ± 4.5	78.9 ± 40.9	2.4 ± 2.3
Antarctic krill, *Euphausia superba*	Whole krill	5	174.3 ± 0.5	4 ± 0.1	0.1	54.2	3.13 ± 0.04	98.0 ± 0.6	275.7 ± 0.5	17.7 ± 0.1
Krill, *Nyctiphanes australis*	Whole krill	5	91.4 ± 1.1	2.8	0.1	35.9	6.6 ± 0.01	40.7 ± 0.2	444.8 ± 2.6	8.0 ± 0.1
Krill, *Euphausia pacifica*	Whole krill	5	62.1 ± 0.6	2.3	0.1	45.2	1.4 ± 0.009	15.6 ± 0.2	293.6 ± 2.3	9.2 ± 0.1
Krill, *Meganyctiphanes norvegica*	Whole krill	10	11.3 ± 8.9	2.2 ± 0.5	0.04 ± 0.02	43.2 ± 2	1.06 ± 0.6	44.6 ± 11.0	90.5 ± 40.8	2.0 ± 0.8
Average among krill	Whole krill	25	76.6 ± 64.1	2.7 ± 0.8	0.08 ± 0.03	44.3 ± 6.6	2.8 ± 2.3	49.1 ± 30.5	255.5 ± 141.6	8.4 ± 6.1

Carbon data for humpback whales are not available
Krill samples were homogenates of 5 animals of each species
Iron data for all species have been discussed in Nicol et al. (2010)

Table 2.3 Summary of dissolved and particulate trace element concentrations in surface waters from the literature (nmol L^{-1}).

Sampling location	**Depth (m)**	**Size partitioning**	**Fe**	**Cd**	**Co**	**P**	**Cu**	**Zn**	**Mn**	**C**	**Reference**
Marguerite Bay, WAP		Dissolved									Hendry et al. (2010)
Ross Sea	0-100	Dissolved		0.34-0.86			0.43-3.3	2.2-8.2	0.33-1.2		Corami et al. (2005)
Ross Sea	0.5-375	Dissolved		0.04-0.73			1.23-2.16	0.24-5.17			Fitzwater et al. (2000)
Ross Sea	0-380	Dissolved					0.5-11.6		0.01-6.6		Grotti et al. (2001)
Weddell Sea	50	Dissolved	2.01						0.34		Westerlund and Öhman (1991)
Atlantic sector	40	Dissolved		0.155-0.905							Löscher et al. (1998)
Atlantic sector	40-100	Dissolved					0.95-6.66	1.7-10.8			Löscher (1999)
Indian-Pacific sector	40	Dissolved		0.25-0.27			1.2-1.4	2.3-2.4			Frew et al. (2001)
Indian-Pacific sector	40	Dissolved	0.1								Bowie et al. (2001)
Southern Ocean	0-20	Dissolved	0.03	0.34	0.02		1.78	1.01	0.08		Cullen et al. (2003)
Ross Sea	0-100	Particulate		0.011-0.097			0.05-0.733	0.2-1.2	19-198		Corami et al. (2005)
Ross Sea	0.5-100	Particulate							0.01-0.17		Fitzwater et al. (2000)
Ross Sea	0-380	Particulate					0.04-1.36		0.01-3.1		Grotti et al. (2001)
Weddell Sea	50	Particulate	2.18						0.022		Westerlund and Öhman (1991)
Atlantic sector	40	Particulate		0.02-0.14							Löscher et al. (1998)
Atlantic sector	40-100	Particulate					0.026-0.222				Löscher (1999)
East Antarctica	0-1	Particulate		0.001-0.018			0.017-0.070	0.020-0.805	0.007-0.141	1170	Lannuzel et al. (2011b)
Amundsen Sea open ocean	8-50	Particulate	0.071-0.66			16.6-44.5			8.81-39.4		Planquette [26]
Southern Ocean	0-20	Particulate	0.26	0.34	0.04		0.38	2.91	0.44		Cullen et al. (2003)
Overall ranges		Dissolved	0.03–2.01	0.04-0.9	0.02		0.43-6.6	0.24-10.8	0.01-6.6		
		Particulate	2.18	0.01-0.14	0.04	16.6-44.5	0.017-1.36	0.02-2.91	0.01-198	1170	

Data from Frew et al. (2001) and Bowie et al. (2001) in the Australasian-Pacific sector are from non-fertilised surface waters

Table 2.4 Trace element concentrations (mean ± standard deviation) in cellular phytoplankton (μmol L^{-1}).

Species	**Algal taxa**	**Sampling location**	**Fe**	**Cu**	**Zn**	**Mn**	**C**	**Reference**
Unknown	Diatoms (Low Fe)	Southern Ocean	45 ± 7		982 ± 235	28 ± 4		Twining and Baines (2004)
	Autotrophic flagellates (Low Fe)	Southern Ocean	143 ± 15		455 ± 74	48 ± 10		Twining and Baines (2004)
	Heterotrophic flagellates (Low Fe)	Southern Ocean	270 ± 50		1615 ± 484	51 ± 8		Twining and Baines (2004)
	Diatoms (High Fe)	Southern Ocean	235 ± 27		1331 ± 350	48 ± 8		Twining and Baines (2004)
	Autotrophic flagellates (High Fe)	Southern Ocean	715 ± 94		971 ± 265	77 ± 11		Twining and Baines (2004)
	Heterotrophic flagellates (High Fe)	Southern Ocean	463 ± 57		2410 ± 643	99 ± 18		Twining and Baines (2004)
Thalassiosira pseudona	Diatom	Sargasso sea		21.4 ± 6.5			13.9 ± 0.26	Annett et al. (2008)
	Diatom	Sargasso sea		56.6 ± 5.1			12.7 ± 0.010	Annett et al. (2008)
Thalassiosira oceanica	Diatom	Sargasso sea		3.43 ± 0.27			10.2 ± 1.1	Annett et al. (2008)
	Diatom	Sargasso sea		79.3 ± 4.8			17.0 ± 1.2	Annett et al. (2008)
Skeletonema menzeli	Diatom	Sargasso sea		4.75 ± 0.57			10.9 ± 0.72	Annett et al. (2008)
	Diatom	Sargasso sea		33.8 ± 11			11.1 ± 0.97	Annett et al. (2008)

Twining and Baines (2004) - Concentrations prior to Fe fertilisation are Low Fe, and following Fe fertilisation are High Fe
Annett et al. (2008) - We used the highest and lowest Cu concentrations measured for each species of phytoplankton and its corresponding C concentration

Table 2.5 Summary of trace element concentrations in Antarctic krill and marine mammals from the literature (mg kg^{-1}).

Species	**Sample type**	**n**	**Fe**	**Cd**	**Co**	**Cu**	**Zn**	**Mn**	**Reference**
Antarctic krill, *Euphausia superba*	Whole	152	0.8 – 1.45	0.2 – 0.48		3.2 – 8.1	2.2 – 4.9	0.14 – 0.4	Yamamoto et al. (1987)
	Whole	-	52.2 – 64.2	0.59 – 0.78	0.064 – 0.074	69.9 – 71.2	59.6 – 66.0	3.82 – 4.2	Barbante et al. (2000)
Adelie penguin, *Pygoscelis adeliae*	Muscle	10	109 – 204	0.04 – 0.46		2.2 – 3.05	18.9 – 27.2	0.21 – 0.35	Honda et al. (1987)
	Liver	10	233 – 1670	0.99 – 8.46		3.26 – 6.06	31.9 – 73.4	1.57 – 2.9	Honda et al. (1987)
	Kidney	10	162 – 360	23.8 – 93.4		2.89 – 4.51	29.6 – 71.4	0.95 – 2.18	Honda et al. (1987)
	Whole	10	68.7 – 163	0.33 – 1.07		1.89 – 2.2	27.1 – 35.7	0.6 – 1.02	Honda et al. (1987)
Southern minke whale, *Baleoptera acutorostrata*	Muscle	37	10.5 – 67.5	0.01 – 0.2		0.42 – 0.78	6.9 – 25.7	0.6 – 0.19	Honda et al. (1987)
	Liver	37	35.2 – 4482	2.32 – 41.7		4.25 – 11.2	30.2 – 70.1	1.6 – 4.89	Honda et al. (1987)
	Kidney	37	20.2 – 114	3.5 – 85		1.87 – 3.75	23.3 – 60.1	0.61 – 1.37	Honda et al. (1987)
	Whole	37	12.3 – 149	0.1 – 0.9		0.59 – 1.1	14.6 – 50.4	0.18 – 0.4	Honda et al. (1987)
Weddell seal, *Leptonychotes weddell*	Muscle	2	237 – 267	0.01 – 0.03		0.85 – 1.02	33.7 – 39.6	0.13 – 0.14	Honda et al. (1987)
	Liver	2	389 - 940	0.96 – 1.31		15.0 – 25.8	41.7 – 47.0	1.80– 1.86	Honda et al. (1987)
	Kidney	2	159 – 618	2.89 – 9.93		5.12 – 11.0	27.4 – 30.7	0.9 – 1.12	Honda et al. (1987)
	Whole	2	141 - 229	0.05 – 0.1		1.08 – 1.36	19.7 – 20.1	0.15 – 0.2	Honda et al. (1987)
Chinstrap penguin, *Pygoscelis antarctica*	Faeces	32	-	1.23 – 3.48		128.1 - 372.4	94.5 – 354.71		Espejo et al. (2014)
Gentoo penguins, *Pygoscelis papua*	Faeces	40	-	1.23 – 3.58		73.2 -308	110.1 - 430.8		Espejo et al. (2014)
Crabeater seal, *Lobodon carcinophagus*	Muscle	27	0.3 – 0.7	0.01-0.39	0.06 – 0.13	2.7-4.3	57 - 133	0.17 – 0.34	Szefer et al. (1994)
	Liver	27	3.0 – 28.0	4.6 – 38.5	0.1 – 0.2	42-105	89 - 230	9.5 – 17.3	Szefer et al. (1994)
	Kidney	27	0.3 – 0.69	14.3 - 90	0.17 – 0.3	18.9 – 39.5	80 - 162	2.0 – 5.0	Szefer et al. (1994)
Leapord seal, *Hydrurga leptonyx*	Muscle	3	0.57 – 0.85	0.03 – 0.1	0.07 – 0.12	2.5 – 5.4	79 - 91	0.11 – 0.14	Szefer et al. (1994)
	Liver	3	2.1 – 3.64	4.0 – 8.5	0.12 – 0.16	98 – 116	145 - 221	13.9 – 15.0	Szefer et al. (1994)
	Kidney	3	0.5 – 0.81	15.7 – 35.9	0.20 – 0.23	22.5 – 43.8	102 – 147	2.1 – 4.7	Szefer et al. (1994)
	Stomach content	4	0.57 – 0.81	0.03 – 0.06	0.06 – 0.1	13.3 – 16.4	61 - 87	0.22 – 0.25	Szefer et al. (1994)
Weddell seal, *Leptonychotes weddell*	Muscle	2	0.87 – 1.42	0.01 – 0.06	0.08 – 0.12	2.1 – 3.1	104 – 133	0.24 – 0.4	Szefer et al. (1994)
	Liver	2	1.09 – 3.57	0.8 – 5.6	0.14 – 0.19	28.0 – 87.1	147 – 189	10.4 – 15.4	Szefer et al. (1994)
	Kidney	2	0.33 – 0.51	6.9 – 44.5	0.19 – 0.21	21.7 – 24.5	88 - 158	2.1 – 4.4	Szefer et al. (1994)

Trace element concentrations in marine mammals from Honda et al. (1987) are in mg/ wet kg. All other trace element concentrations are in mg/ dry kg

Table 2.6 Trace metal: carbon ratios (mean ± standard deviation) in whale faeces, whale muscle and Antarctic krill (μmol mol^{-1}, C:P in mol mol^{-1}). For comparison, Redfield ratio of C:P is 106:1 mol:mol.

Species	**Sample type**	**n**	**Fe:C**	**Cd:C**	**Co:C**	**Cu:C**	**Zn:C**	**Mn:C**	**C:P**	**Reference**
Pygmy blue, *Baleoptera musculus brevicauda*	Faeces	7	76.5 ± 14.3	4.3 ± 0.98	0.5 ± 0.2	342.7 ± 125.7	644 ± 120.9	19.7 ± 9.6	5.4 ± 1	This study
Blue, *Baleoptera musculus*	Faeces	15	206.8 ± 148.5	17.2 ± 5.8	1.3 ± 1.2	262 ± 82.2	493.7 ± 250.4	41.7 ± 17.8	5.1 ± 1.7	This study
	Muscle	1	21.3	0.005	0.04	3.2	14.9	0.1	426.7	This study
Fin, *Baleoptera physalus*	Faeces	2	230.2 ± 48.2	20.3 ± 7	1.9 ± 1.3	247.8 ± 5.7	334.5 ± 44.1	29.9 ± 7.6	4.7 ± 0.1	This study
	Muscle	1	91.04	0.009	0.04	3.2	35.6	1.9	227.3	This study
Sperm whale, *Physeter macrocephalus*	Faeces	1	467.4	176.4	1.3	887.7	1405.1	60.3	129.9	This study
Average among whales	Faeces	27	182.7 ± 142.2	20.2 ± 33.5	1.15 ± 1.1	308.5 ± 154.5	559.6 ± 281.2	35.3 ± 18.3	10.2 ± 25	This study
	Muscle	2	56.1 ± 49.3	0.007 ± 0.003	0.02 ± 0.03	1.9 ± 1.8	25.3 ± 14.7	1.02 ± 1.3	327.1 ± 141	This study
Antarctic Krill, *Euphausia superba*	Whole krill	5	69.04	0.76	0.04	34.03	93.4	7.15	44.2	This study
Krill, *Nyctiphanes australis*	Whole krill	5	54.3	0.8	0.06	21.4	226.7	4.9	13.9	This study
Krill, *Euphausia pacifica*	Whole krill	5	23.9	0.5	0.03	6.6	118.6	4.5	84	This study
Krill, *Meganyctiphanes norvegica*	Whole krill	10	4.0 ± 4.8	0.5 ± 0.06	0.02 ± 0.01	20.3 ± 7.6	30.8 ± 16.2	0.76 ±0.03	206.5	This study
Average among krill	Whole krill	25	32.1 ± 29.5	0.6 ± 0.2	0.03 ± 0.01	20.5 ± 10.4	100 ± 81	3.6 ± 2.8	93.4 ± 74.6	This study
Phytoplankton	Diatoms		6	3.4	67.8					Twining and Baines (2004)
	Autotrophic flagellates		8.7	2.7	22.2					Twining and Baines (2004)
	Heterotrophic flagellates		14.1	3	46.9					Twining and Baines (2004)
	Diatoms					0.335 ± 0.030				Annett et al. (2008)
	Diatoms					4.46 ± 0.40				Annett et al. (2008)

All data from Twining and Baines (2004) are from low Fe conditions
For data from Annett et al. (2008) we used the lowest and highest Cu:C ranges

2.4.2 Metal: Carbon and carbon to phosphorus ratio

When normalised to C, the concentration of Cd, Cu, Co, Mn and Zn were higher in krill tissue compared to whale muscle, whereas Fe was higher in whale muscle compared to krill tissue (Table 2.6 and Figure 2.1). All metal to C ratios were higher in whale faeces compared to whale muscle. When normalised to P, the C content was highest in whale muscle followed by krill tissue and lastly whale faeces (Table 2.6 and Figure 2.2). Redfield C:P molar ratio of 106:1 mol:mol is typical of phytoplankton (Redfield 1958). Here, whale faeces and krill tissue are below the C:P Redfield ratio and whale muscle are higher.

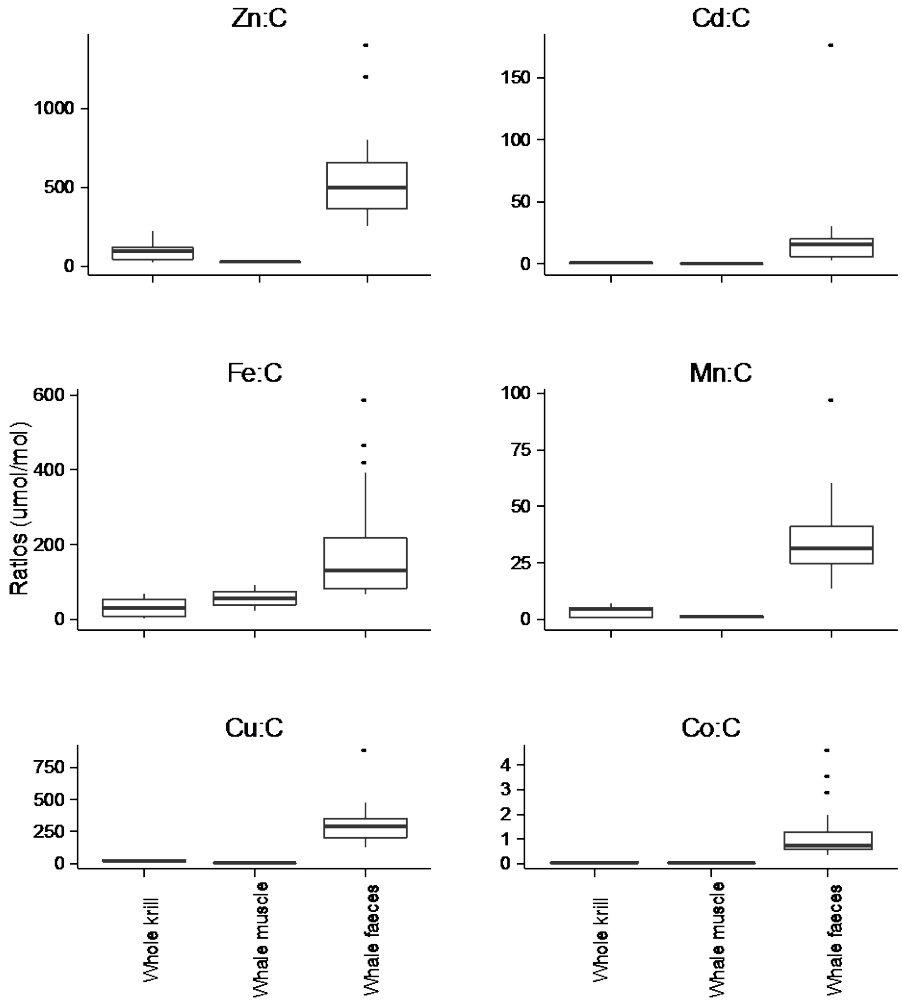


Figure 2.1 Metal to carbon ratios in krill and whales (μmol mol^{-1}). Data points above the third quartile for whale faeces are 3 or more times higher than the interquartile range.

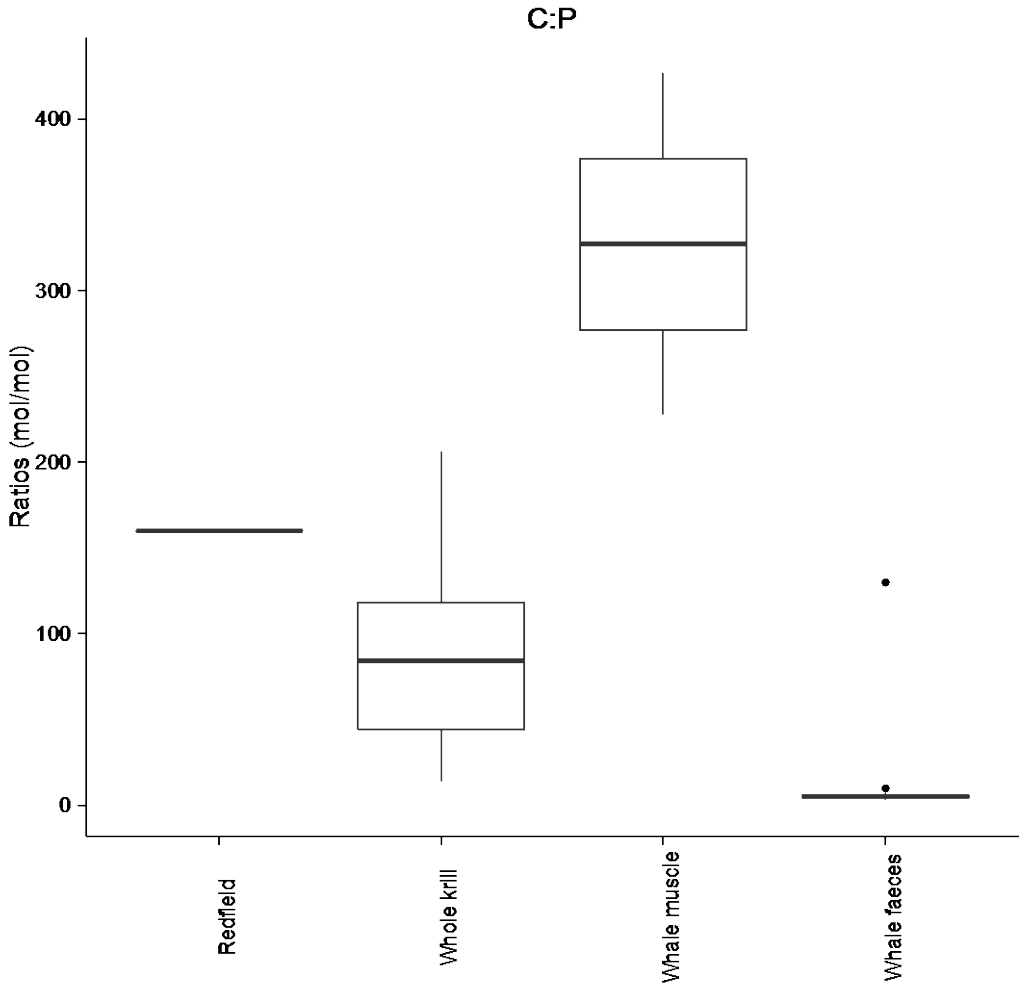


Figure 2.2 Carbon to phosphorus ratio in krill and whales (mol mol^{-1}). Data point above the third quartile for whale faeces is 3 or more times higher than the interquartile range.

2.5 Discussion

2.5.1 Comparison to published analyses

The concentrations of trace elements in krill from this study were within the reported ranges for the Antarctic krill (Table 2.5) (Yamamoto et al. 1987, Barbante et al. 2000). For whale muscle, the concentration of Cd, Cu and Zn were similar to published values from other Southern Ocean marine mammals: Crabbeater seal (*Lobodon carcinophagus*), Leopard seal (*Hydrurga leptonyx*), and Weddell seal (*Leptonychotes weddellii*) (Table 5) (Szefer et al. 1994).

Most studies investigating trace element concentration in marine vertebrates have used liver or kidney tissue as a means of quantifying the bioaccumulation of metal contaminants. However, as liver plays an important role in accumulation and detoxification of elements, it is expected that the concentration of elements in liver and kidney would not be comparable with trace element concentrations in muscle samples analysed in this study (Honda et al. 1987). Unfortunately we did not have any samples from other whale tissue to compare with the literature values. The concentration of Fe, Mn, Zn, Cd and Cu in whale muscle from this study was much higher than published muscle concentrations of the Southern minke whale (*Baleoptera acutorostrata*) (Table 2.5) (Honda et al. 1987). In whale faeces, the concentration of Cd, Cu and Zn were higher than published values for faeces from Antarctic chinstrap penguins (*Pygoscelis antarctica*) (Table 2.5) (Espejo et al. 2014). To the best of our knowledge, there are no other studies that have reported trace element concentrations in faecal matter from Antarctic vertebrates.

2.5.2 Antarctic krill and baleen whales as sources of trace elements to ocean surface waters

Iron has been demonstrated to be the primary factor controlling marine primary productivity in one third of the world's oceans, including the climatically important Southern Ocean. Iron-containing proteins are essential for photosynthetic and respiratory electron transport (Sandmann 1985), and Fe been demonstrated to limit the growth rates of the diatom *Thalassiosira weissflogii* and the dinoflagellate *Prorocentrum minimum* when the unchelated Fe concentrations in seawater fall below 0.1 nmol L^{-1} (Sunda and Huntsman 1997). This is further supported by the 100-fold increase in diatom concentrations following natural and artificial Fe-fertilization experiments in HNLC surface waters (see Boyd et al. (2007) and de Baar et al. (2005) for a

synthesis).

Dissolved and particulate Fe concentration in surface seawater of HNLC regions is typically less than 1 nmol L^{-1} (de Baar et al. 1995, Bowie et al. 2001, Cullen et al. 2003). This micronutrient can be passively scavenged onto particles or actively taken up by organisms. Nicol et al. (2010) indicated that the Southern Ocean krill population could contain approximately 24% of the total Fe in the surface waters within its range, and whale faecal Fe content (145 ± 133.7 mg kg^{-1}) was approximately ten million times that of Southern Ocean surface seawater concentrations. Here we confirm that krill concentrate the Fe derived from phytoplankton into its tissue, with the Fe:C ratio in krill 3 times higher than the averaged published value for Southern Ocean phytoplankton in low Fe conditions (Table 2.6). In whale muscle, the Fe:C ratio was almost double that of krill and in whale faecal matter it was over 5 times higher than krill tissue. This indicates that whales are concentrating the carbon and actively defecating the Fe.

Manganese is also a crucial trace element in seawater, and it is required by the water oxidizing complex of photosystem II in phytoplankton (Morel et al. 2003). The concentration of Mn in Southern Ocean surface waters is typically low (dissolved and particulate 0.02 – 6.77 nmol L^{-1}, but 19.33 – 199.2 nmol L^{-1} in the Ross Sea, Corami et al. 2005, and 8.81 – 39.4 nmol L^{-1}, particulate only, in the Amundsen Sea, Planquette et al. 2013). However published average cellular concentrations of Mn in diatoms from low Fe waters in Southern Ocean were between 200 and 2 million times higher than surface water concentrations suggesting that phytoplankton is enriched in Mn. Manganese is also an essential element for metabolism in crustaceans (Baden and Eriksson 2006). Accordingly, krill tissue showed even higher concentrations of Mn (8.4 ± 6.1 mg kg^{-1}), which is over 300,000 times higher than typical HNLC seawater concentrations of 0.52 nmol L^{-1} (dissolved and particulate) (Cullen et al. 2003). Whale muscle had low concentrations of Mn (2.4 ± 2.3 mg kg^{-1}), and lower Mn:C ratio compared to whale faeces. This is because Mn is not assimilated and consequently is often used as a measure of assimilation efficiency in marine mammals (Fadely et al. 1990). As a result, and similar to Fe, whales defecate most of their dietary Mn as demonstrated by high Mn content in their faeces (27.3 ± 16.3 mg kg^{-1}) compared to their muscle (2.4 ± 2.3 mg kg^{-1}).

The Zn, Co and Cd concentrations in Southern Ocean surface waters are low (0.24 – 9.4 nmol L^{-1},0.00006 pmol L^{-1} and 0.04 – 0.905 nmol L^{-1}, respectively – Table 2.2), however, these elements are essential cofactors in metalloenzymes in marine phytoplankton. All marine phytoplankton have adapted to limitations of CO_2 diffusion in water by evolving carbon concentrating mechanisms (CCMs) to support photosynthetic carbon fixation (Reinfelder 2010). The CCM catalyses the equilibrium between bicarbonate (HCO_3^-) and CO_2 using the Zn metalloenzyme carbonic anyhydrase (Morel and Price 2003). Under Zn limitation, the carbonic anhydrase can function with Co or Cd instead of Zn (Lane and Morel 2000). Therefore the ability of marine phytoplankton to acquire CO_2 also depends on the availability of Zn, Co and Cu in surface waters.

The mean cellular concentrations of Zn in diatoms vary by 2 orders of magnitude (3.43 – 982 μmol L^{-1} – Table 2.3); however diatoms show cellular accumulation of Zn, with concentrations between 1000 to 100,000 times higher than seawater (Table 2.4). Zinc is then further concentrated in krill tissue (275.4 ± 137.2 mg kg^{-1}). Whale muscle was relatively low in Zn (74.9 ± 40.9 mg kg^{-1}) compared to krill tissue, and Zn:C ratios were lower in whale muscle compared to whale faeces suggesting the low requirement of whales on this element. As such, most of the Zn is released through whale faecal matter (621.5 ± 432.9 mg kg^{-1}).

Cobalt and Cd were present in very low concentrations in krill tissue (0.08 ± 0.03 mg kg^{-1} and 2.8 ± 0.7 mg kg^{-1}, respectively) suggesting that relative to other trace elements measured in this study, krill may have little use for Co and Cd. When normalised to C, Co and Cd were higher in phytoplankton compared to the average among krill (Table 2.6). Similarly Co and Cd were scarce in whale muscle (0.04 ± 0.04 mg kg^{-1} and 0.1 ± 0.2 mg kg^{-1}, respectively). When normalised to C, Co and Cd were lower in whale muscle compared to whale faeces, indicating that these elements are expelled through their faecal matter (0.94 ± 0.87 mg kg^{-1} and 34.7 ± 88.9 mg kg^{-1}, respectively). Interestingly, the concentration of Cd in sperm whale faeces was much higher compared to other species of whales in this study (575 mg kg^{-1}), which may reflect the different diet of this species. Sperm whales in the Southern Ocean predominantly consume squid which may predate on Antarctic krill (Nemoto et al. 1988).

Copper is one element that shows clear differential uptake and utilization across the food web compared to other elements in this study. Copper concentration in seawater is low (dissolved and particulate 0.48 – 12.96 nmol L^{-1} - Table 2.2) and is little concentrated by phytoplankton (3.48 – 79.3 μmol L^{-1}) (Annett et al. 2008), which appear to have little physiological use for it. Studies have demonstrated that Cu is toxic to the dinoflagellate *Gonyaulax tamarensis* and the diatom *T. pseudonana*, and is able to decrease their growth at only a few pmol L^{-1} (Sunda and Guillard 1976, Anderson and Morel 1978). Krill, like most crustaceans however, require Cu, as it is an essential element in their respiratory pigment; hemocyanin (Spicer and Saborowski 2010). Accordingly, krill tissues show a marked bio-concentration of Cu (49.1 ± 30.5 mg kg^{-1} – Table 2.5, and Cu:C 20.5 ± 10.4 μmol mol^{-1} - Table 2.6), 100,000 times higher than Southern Ocean surface waters and over 1.5 million times higher than that measured for Southern Ocean diatoms. Whale muscle was relatively low in Cu (5.3 ± 4.5 mg kg^{-1}) compared to their prey, which reflects the lower physiological dependency of mammals on this element. Consequently, whale faeces contained high levels of Cu (1635 5.3 mg kg^{-1} in sperm whales, 253.5 ± 100.4 mg kg^{-1}, all other species), and higher Cu:C ratio compared to whale muscle, suggesting that whales take up relatively little Cu from their diet.

Phosphorus is an essential nutrient required for structural and functional components of all organisms. Despite a high range, the mean C:P ratio in whale muscle from our study was 30 times higher than mean whale faeces ratio and 3 times higher than the Redfield ratio (Figure 2.2), indicating that whales are actively storing the P in their muscle. When nutrients are not limiting, the C:P ratio in most phytoplankton is 106:1 (Redfield 1958). When P is scarce, phytoplankton have been demonstrated to reduce their cellular P requirements by substituting phospholipids for non-P membrane lipids (Van Mooy et al. 2009). In the Southern Ocean, surface water phosphate concentrations (16.6 – 44.5 nmol L^{-1}, Planquette et al. 2013) are much higher than the other elements we report here. Despite this, the concentration of P in krill was over 30 million times higher than median surface water concentrations (28,304.1 ± 23,286.7 mg kg^{-1}). Whales concentrate the P from krill for biochemical processes.

Our results suggest that Antarctic krill and whales may be a key part of marine biogeochemical cycling and act as a source of essential and limiting trace elements to phytoplankton in surface waters of the Southern Ocean. Krill and whales are long-lived, actively swimming animals that do not undergo any form of dormancy. As such, the large stock of krill can act as a mechanism of retaining trace elements in the surface waters whereas whales concentrate certain elements required for physiological processes from the krill, but actively defecate other elements that can be used for phytoplankton production. In addition, krill are capable of absorbing elements such as fluorine directly from seawater suggesting that they can concentrate some elements despite their scarcity in surface waters (Nicol and Stolp 1991).

2.5.3 Ecological importance of whales – past, present and future

The loss of large predators from marine ecosystems has the potential to affect marine biogeochemistry, and consequently marine primary productivity and carbon sequestration (Nicol et al. 2010, Pershing et al. 2010, Wing et al. 2014). Because of their vast size and huge consumption of krill, blue and fin whales would have been the dominant krill consumers in the Southern Ocean before the era of commercial whaling and thus would have been the significant contributors to ocean nutrient recycling. Although their large size acts as a carbon store, their major role is in how they affect the recycling of critical elements, and it is the availability of these elements that affects the ocean's ability to sequester carbon. Consequently it has been suggested that the efficiency of recycling and supply of essential nutrients to surface waters has diminished in the Southern Ocean due to massive reductions in whale numbers through commercial whaling (Smetacek 2008, Lavery et al. 2010, Lavery et al. 2014).

The pre-exploitation population of Antarctic blue whales was estimated to be between 202,000 to 311,000 individuals and was expected to have exported approximately 72,172 tons C yr^{-1} (Branch et al. 2004, Pershing et al. 2010). Current estimates of Antarctic blue whales are approximately 4,727 individuals, less than 2% of mean pre-exploitation levels (Branch et al. 2004, Pershing et al. 2010), with a predicted recovery rate of 8.2% per year (International Whaling Commission 2000). There is no reliable data on pygmy blue whale abundances. Fin whales are thought to be more abundant and their numbers may be increasing; however, current estimates of population sizes are not available. Many humpback whale populations are

recovering quickly but their current numbers are still considerably below pre-exploitation population sizes. The recovery of the great whales could increase the spatial extent of productive regions in the Southern Ocean through the recycling of essential nutrients to surface layers from their faecal matter (Smetacek 2008, Pershing et al. 2010).

2.6 Conclusion

There is accumulating evidence of the role of whales in the ocean nutrient cycling and their importance relative to their abundance (see Lavery et al. (2010), Nicol et al. (2010), Pershing et al. (2010), Lavery et al. (2014), Roman et al. (2014), Wing et al. (2014) for synthesis). Our results show that krill can act as a reservoir of essential trace elements in surface waters, and whales can release these stored elements through feeding and defecation. This study further extends the role of larger animals as important components of ocean biogeochemical cycling for a range of elements. To fully understand the role of large marine mammals in ocean biogeochemical cycling future studies will have to determine the bioavailability of the elements contained in whale faeces, and to quantify the combined effects of, nutrient recycling in the surface layer, the effects of nutrient scavenging from deep water and biogenic turbulence caused by vertically migrating whales.

2.7 Acknowledgements

We would like to thank Dr Pier van der Merwe and Dr Ashley Townsend for assistance in the laboratory and Dr Marion Fourquez and Dr Simon Jarman for their comments and suggestions during the development of this project and manuscript. Samples were collected under the Australian Antarctic Animals Ethics permit for project 2926. No whales were killed in the pursuit of this research.

3 A preliminary model of iron fertilisation by baleen whales and Antarctic krill in the Southern Ocean: sensitivity of primary productivity estimates to parameter uncertainty

This chapter has been published:

Ratnarajah L, Melbourne-Thomas J, Marzloff MP, Lannuzel D, Meiners KM, Chever F, Nicol S, Bowie AR. (2016a) A preliminary model of iron fertilisation by baleen whales and Antarctic krill in the Southern Ocean: sensitivity of primary productivity estimates to parameter uncertainty. Ecological Modelling 320: 203 – 212 doi:10.1016/j.ecolmodel.2015.10.007

3.1 Abstract

Large marine animals may play a crucial role in storing and recycling bioavailable iron in surface waters by consuming iron-rich prey and subsequent defecation of iron that is excess to their requirements. This biological recycling of iron could enhance primary productivity in iron-limited waters. However, quantifying the effects of marine animals on ocean primary productivity remains challenging because of a limited understanding of the key biogeochemical processes involved. In this paper, we develop a preliminary model that explores these uncertainties and examines the potential effects of historical populations of blue, fin and humpback whales, and the biomass of Antarctic krill required to support the whale populations, on primary productivity in the Southern Ocean.

Our results suggest that, despite conservative estimates for key processes in our model, pre-exploitation populations of blue whales and, to a lesser extent fin and humpback whales, could have contributed to iron recycling, resulting in enhanced phytoplankton production in iron-limited Southern Ocean waters. Iron-rich defecation by un-exploited whale populations in the Southern Ocean, and the biomass Antarctic krill required to support them, could have resulted in a contribution to primary productivity of between 1.5 x 10^{-4} to 23.4 g C m^{-2} yr^{-1} (blue whales), 1.4 x 10^{-4} to 13.9 g C m^{-2} yr^{-1} (fin whales), and 2.4 x 10^{-5} to 1.7 g C m^{-2} yr^{-1} (humpback whales). However, only when all parameter estimates are at their upper limits does there appear to be this significant role for whales in enhancing primary productivity, and thus we need to assess the likelihood of these values arising.

The high degree of uncertainty around the magnitude of these increases in primary productivity is mainly due to our limited quantitative understanding of key biogeochemical processes. To reduce uncertainty regarding the effect of whales on Southern Ocean primary productivity, future research will need to refine our understanding of five influential model parameters: iron content in krill; krill consumption rates by whales; persistence of whale faecal iron in the photic zone; bioavailability of this retained iron; and the carbon-to-iron ratio of phytoplankton.

3.2 Introduction

Large regions of the Southern Ocean are characterised by low phytoplankton biomass despite high concentrations of major nutrients (e.g. nitrate, phosphate and silicate), and have been characterised as High Nutrient Low Chlorophyll (HNLC) waters (Moore and Abbott 2000, Boyd et al. 2007). Multiple artificial iron fertilisation experiments have demonstrated that the major factor responsible for limiting the accumulation of phytoplankton in HNLC waters is the availability of the essential trace element iron (see de Baar et al. 2005 and Boyd et al. 2007 for synthesis). Natural sources of iron into the upper ocean are from atmospheric dust depositions (Boyd et al. 2004, Cassar et al. 2007), shelf sediments (Sedwick et al. 2008, Bowie et al. 2009), melting icebergs (Smith et al. 2007, Lin et al. 2011) and sea ice (Sedwick and Di Tullio 1997, Lannuzel et al. 2007), and mediated through upwelling and vertical mixing. These external sources of iron to HNLC waters are typically very low (Boyd et al. 2000, Bowie et al. 2001, de Baar et al. 2005); consequently, biological recycling could increase the availability of iron to phytoplankton (Figure 3.1).

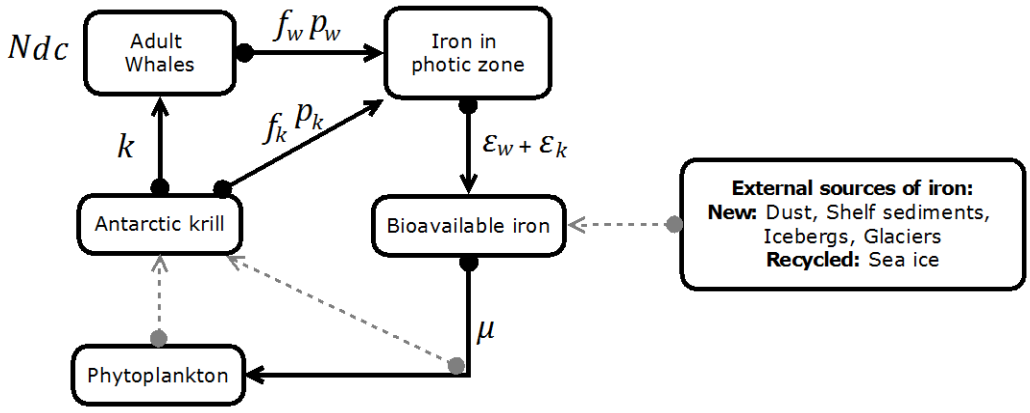


Figure 3.1 Conceptual model of the biological recycling of iron in the Southern Ocean. Solid black lines represent interactions considered in this model. Dashed grey lines represent uncertain interactions not considered in this model. k is the concentration of iron in krill tissue (in mg kg^{-1}), N are the pre-exploitation population estimates for whales, c is the daily consumption rate (in kg day^{-1}), d is the feeding duration (in days), f is the proportion of iron defecated, p is the persistence of defecated iron, ε is the bioavailability of faecal iron and u is the carbon-to-iron

ratio in phytoplankton (in mol mol^{-1}). *w* and *k* subscripts next to parameters f, p and ε stand for whales and krill respectively.

Marine animals may play a crucial role in storing and recycling iron in surface waters through their iron-rich diet, and subsequent defecation (Smetacek 2008, Lavery et al. 2010, Nicol et al. 2010, Roman and McCarthy 2010, Lavery et al. 2014, Ratnarajah et al. 2014, Roman et al. 2014, Wing et al. 2014). Increased persistence and availability of iron in surface waters could enhance overall marine primary productivity in HNLC waters. However, the contribution of Antarctic krill (*Euphausia superba*) and whales to iron recycling is difficult to study, let alone quantify, *in situ*. Consequently, our current understanding of iron recycling by Antarctic krill and whales is limited. Here, we have collated published information about key processes and measurements to estimate the effects of Antarctic krill and baleen whales on primary productivity in the Southern Ocean, and identify major sources of uncertainty in our understanding of iron recycling by marine animals in the Southern Ocean.

Lower trophic level crustaceans are capable of taking up iron from their diet, and from the surrounding water through their gills or other permeable cuticles (Marsden and Rainbow 2004). The iron is then either stored in their body tissue, cuticle and ventral caeca (gut) for physiological requirements or excreted through their antennal glands, gills, guts and moulting (Marsden and Rainbow 2004). Specifically, passive diffusion contributes to fluoride uptake in Antarctic krill (Nicol and Stolp 1991), but there is no knowledge of similar uptake through diffusion of other elements, such as iron. Recent studies, however, have demonstrated that Antarctic krill could play a key role in storing and recycling iron in the Southern Ocean (Tovar-Sanchez et al. 2007, Nicol et al. 2010, Ratnarajah et al. 2014).

During their feeding season in the Southern Ocean, balaenopterid whales feed on iron-rich Antarctic krill as their main dietary source (Lockyer 1981, Nicol et al. 2010). Mammals require iron for the production of red blood cells (haemoglobin), the oxygen storage protein in muscles (myoglobin), and the iron containing centres in many enzymes (Ordway and Garry 2004, Ganz and Nemeth 2006). However mammals are only able to excrete assimilated iron by shedding of intestinal and skin cells, and through minor blood loss in the intestine (Ganz and Nemeth 2006).

The limited ability of mammals to excrete iron means that the iron absorbed during the growth phase of the whale is retained until adulthood and recycled for future use (e.g. the recycling of iron from senescent red blood cells for the production of new red blood cells, Ganz and Nemeth 2006), and excess iron not used in these processes is defecated. Consequently, their buoyant, fluid-like faeces of baleen whales is iron-rich and could act a fertiliser for phytoplankton growth; with iron concentrations over 10 million times higher than Antarctic surface waters (Nicol et al. 2010, Ratnarajah et al. 2014).

Here, we develop a preliminary model for iron recycling by historical Southern Ocean population levels of blue (*Baleoptera musculus*), fin (*Baleoptera physalus)*, and humpback (*Megaptera novaeangliae*) whales and the biomass of Antarctic krill required to support each whale population. In this model we only consider the component of the ecosystem that comprises historical populations of whales, the krill consumed by these whales (we do not consider the entire, unknown, historical population of krill in the Southern Ocean), and the phytoplankton biomass that might be stimulated through iron recycling of these two components. The objective of our study was to analyse the influence of parameter uncertainty on the estimated contribution of iron recycling by whales and Antarctic krill to primary productivity, and to identify the most influential and uncertain parameters that could usefully be targeted as priorities for future data collection. We use a local sensitivity analysis (*sensu* Cariboni et al. 2007) in which parameters are varied individually from their baseline (hereafter referred to as mean) values, to assess changes in estimates of primary productivity. To our knowledge, this study is the first to consider uncertain quantification of key biogeochemical processes involved in biological recycling of iron.

3.3 Methods

3.3.1 Model description

We modelled iron recycling in the Southern Ocean, which we defined as the area south of 60°S latitude that encompasses 2 x 10^7 km^2 around Antarctica. We used published estimates of historical blue, fin and humpback whale populations (*N*) in the Southern Ocean, feeding duration in polar waters (*d*, in days per year), species-specific daily consumption rates (*c*, in kg day^{-1}), and a conversion factor from wet weight to dry weight (α), to determine the biomass of Antarctic krill (*B*, in kg dry weight yr^{-1}) required to support pre-exploitation levels of whales (Equation 1).

$$B = Nd\ (\alpha c) \qquad \text{Equation 1}$$

We then multiplied the biomass of Antarctic krill (*B*, in kg dry weight yr^{-1}) with the total iron content in Antarctic krill tissue (*k*, in g kg^{-1} dry weight) to determine the amount of iron consumed by whales (W_{fe}, in g dry weight yr^{-1}) during their time in the Southern Ocean (Equation 2).

$$W_{fe} = Bk \qquad \text{Equation 2}$$

To determine the amount of iron available for phytoplankton (P_w, in g dry weight) we assume that whales defecate daily during their feeding season, and a proportion of iron consumed is defecated (f_w). A proportion of this defecated iron persists in the photic zone (p_w), and a lesser proportion of this retained faecal material is bioavailable (ε_w) for phytoplankton uptake (Equation 3). M_{Fe} is the molecular mass of iron (55.845 g mol^{-1}) to convert units into mol.

$$P_w = \frac{W_{fe} f_w p_w \varepsilon_w}{M_{fe}} \qquad \text{Equation 3}$$

Similarly, iron rich-defecation by Antarctic krill could also stimulate primary productivity. To determine the amount of iron available for phytoplankton (P_k, in g dry weight), we assume that the biomass of Antarctic krill (*B*) required to support pre-exploitation levels of whales defecate daily during their feeding season. A proportion of iron content (*k*) is defecated (f_k). We assume

that Antarctic krill could obtain iron through diffusion in addition to their diet to sustain their metabolic demand. A portion of the defecated iron persists in the photic zone (p_k), and a lesser proportion of this retained faecal material is bioavailable (ε_k) for phytoplankton uptake (Equation 4).

$$P_k = \frac{Bkf_k p_k \varepsilon_k}{M_{fe}} \qquad \text{Equation 4}$$

We then used published carbon-to-iron ratios (u) from laboratory cultures and uptake ratios from field investigations to determine the potential effect of whale recycling of iron on Southern Ocean primary productivity (PP).

$$PP = \frac{(P_w + P_k) M_C u}{a}$$

$$= \frac{\left\{\frac{Ndk(\alpha c)((f_w p_w \varepsilon_w) + (f_k p_k \varepsilon_k))}{M_{Fe}}\right\} M_C u}{a} \qquad \text{Equation 5}$$

where *a* is the area of the Southern Ocean (as defined above). Our primary productivity model is limited to the iron contribution of the population of a single species of whale and the Antarctic krill biomass required to support it (assuming non-limiting macronutrient and light conditions). Further caveats and assumptions are included in Section 3.5.5.

To determine if iron recycling by whales and Antarctic krill is self-sustaining, we took the ratio (χ) of mean primary productivity estimates due to whale and Antarctic krill (*PP*, Equation 5) for the entire Southern Ocean, to the amount of photosynthetic carbon consumed by the biomass of Antarctic krill (*B*) required to support historical whale populations.

$$\chi = \frac{PP}{Br_k} \qquad \text{Equation 6}$$

Br_k represents the metabolic demand of the biomass of Antarctic krill (in g C yr^{-1}) required to support pre-exploitation levels of whales over the same number of days as the feeding duration of whales. r_k is the consumption rate of Antarctic krill. We calculated r_k as 150 g C day^{-1} per kilogram of krill dry weight, based on an estimate for krill consuming 15% of their body weight daily over summer and a wet weight for an individual krill of 0.486 g (Pakhomov et al. 2002). If χ is less than 1, the system relies on external inputs of iron. If χ is more than 1, there is sufficient recycling of iron by whale and krill for the model system to be self-sustaining (assuming that the excess, stimulated primary productivity is available to the portion of the krill population that we are modelling).

Mean, minimum and maximum values for each parameter in our model are obtained from published data (Table 3.1, and as described below). In our local sensitivity analysis, parameters are varied one by one whilst all other parameters are held at their mean values. We also calculated the absolute minimum (PP_{min}) and the absolute maximum (PP_{max}) contributions to primary productivity for each whale species, using minimum and maximum values for all parameters, respectively. The model and sensitivity analysis were coded in R 3.0.3 (R Core Team 2014), and are provided as supplementary material.

3.3.2 Parameter descriptions

There were 12 parameters in our model. Mean, minimum and maximum parameter estimates for pre-exploitation population estimates, *N*, feeding duration, *d*, conversion factor, α, daily consumption rate, *c*, concentration of iron in krill tissue, *k*, and carbon: iron ratio in phytoplankton, *u*, were derived from the published literature (Table 3.1). Mean proportion of iron defecated by whales, f_w, was based on one study (Table 3.1).

There were five parameters where no empirical measurements are available; proportion of iron consumed and subsequently defecated by krill, f_k, and iron persistence and availability in surface waters in whales and krill (p_w and p_k, and ε_w and ε_k respectively). In cases where parameter values have not been estimated empirically, we derive highly conservative values from available qualitative understanding of the processes. Justifications of parameter estimates are detailed below.

3.3.3 Population estimates

We use pre-exploitation population estimates for blue, fin and humpback whales in the Southern Ocean from the International Whaling Commission (2014), Branch et al. (2004) and Leaper and Miller (2011) to assess the potential contribution of un-exploited Southern Ocean whale populations to primary productivity. Population estimates range between 200,000 – 300,000 individuals for blue whales, 235,000 – 325,000 individuals for fin whales, and 75,000 – 100,000 for humpback whales (N, Table 3.1). Here we use the median population estimate for the un-exploited stock of blue, fin and humpback whales as the mean.

Blue, fin and humpback whale populations are estimated to have declined by 90-99%, as a result of commercial whaling, primarily from the 1920's to the 1950's (International Whaling Commission 2014). We estimated the biomass of krill required to support pre-exploitation levels of whales using historical population estimates of whales, their daily consumption rate and feeding duration in the Southern Ocean from the literature (Eq. 1).

3.3.4 Diet

During the summer months, blue, fin and humpback whales primarily feed in the Southern Ocean, consuming a diet almost exclusively of Antarctic krill (*Euphausia superba*). On average, whales are thought to feed in Antarctic waters for at least three to four months (Lockyer 1981). However inter-annual variability in the seasonal cycle could reduce or increase their feeding times in the Southern Ocean. The breeding season for Southern Hemisphere whales is between June – October, (Clapham 1999), leaving 7 months for migrations south, feeding and migrations north. Based on this we assume that whales feed on krill for 3 – 5 months of the year (d, Table 3.1). Daily krill consumption rates of blue and fin whales used in our model correspond with the mean, minimum and maximum of 8 published estimates, while the mean, minimum and maximum consumption rates of humpback whales were obtained from 3 published estimates as calculated in Lockyer (1981) and Reilly et al. (2004) (c, Table 3.1). We only used whale consumption rates from the Southern Ocean and Antarctic region because the Northern Hemisphere whales are smaller in size than their Southern Hemisphere counterpart (Mackintosh 1965).

All model variables are expressed as dry weights. Mean, minimum and maximum iron concentration in whole krill specimens were sourced from 7 published estimates (Locarnini and Presley 1995, Caroli et al. 1998, Barbante et al. 2000, Deheyn et al. 2005, Palmer et al. 2006, Nicol et al. 2010, Kim et al. 2014), and our preliminary data (*k*, Table 3.1). Krill contain approximately 20% dry tissue by weight (Nicol et al. 1992). We used a conversion factor of 0.23 to convert consumption rates by whales from wet (total) weight to dry weight in our model (α, Table 3.1).

3.3.5 Proportion of dietary iron defecated

Mammals cannot excrete iron through their kidneys; therefore iron that has been ingested, and is in surplus to physiological requirements, passes through the animal and is defecated (Candela et al. 1984, Ganz and Nemeth 2006). Our preliminary model of historical whale populations assumes that the population is "stable" and consists largely of adults. Fully grown adult mammals, which are not building muscle, could defecate over 90% of the iron consumed (Candela et al. 1984). Iron is required for many physiological processes (Ordway and Garry 2004, Ganz and Nemeth 2006), and ingestion and assimilation rates differ between marine mammal species with respect to their weight and their migration or physiological status (pregnant or lactating) (Das et al. 2003). Because majority of the individuals in a "stable" population are adults, we assume that 80% of the iron consumed by adult whales is defecated as the mean, with a minimum of 70% and a maximum of 90% (f_w, Table 3.1). These differences also take into account the small proportion of pregnant and lactating females and weaning calves to the overall proportion of adult whales.

We used similar estimates for krill faecal material, as there is no information on the requirement of iron by crustaceans, which would affect the amount of iron they defecate (f_k, Table 3.1). Crustaceans do not contain haemoglobin or myoglobin, however they may have a requirement for iron-containing centres in enzymes.

3.3.6 Phytoplankton uptake

Not all iron in the ocean is bioavailable (i.e. usable for photosynthesis) and there is no information on the persistence and bioavailability of faecal iron in the photic zone. For whales, we assume that between 25 – 75% of the iron defecated by whales persists, and is soluble within the photic zone (p_w, Table 3.1). Based on the diving behaviour of 7 blue whales and 15 fin whales off the coast of California, USA, and Mexico, whales dive to a depth of 113.1 ± 64.3 m for 6.6 ± 2.3 minutes and 78.1 ± 32.1 m for 5.5 ± 1.6 minutes for blue and fin whales respectively (Croll et al. 2001). These dive depths are still within the euphotic zone; therefore we expect that whales defecate within the euphotic zone. Of the faecal material that is retained, we assume that 25 – 75% of the defecated iron is bioavailable for phytoplankton uptake (ε_w, Table 3.1). Even though whales are present mostly at the surface or diving within the euphotic zone, we chose these wide ranges due to the lack of information on the sinking rate and size fractionation of whale faecal material, and the limited understanding of iron bioavailability in the ocean (Geider 1999).

Dietary iron in whale gut is present as ferrous iron (Fe^{2+}) (Naikare et al. 2006), which is assumed to be a more bioavailable form in seawater. However, in these waters Fe^{2+} estimated to have a half-life of less than 1 hour for oxidation to Fe^{3+} (Millero et al. 1987). Despite this fast oxidation rate, Fe^{2+} was still found to be the dominant species present in seawater on day 12 and 13 following iron fertilisation in the Southern Ocean (SOIREE) possibly due to a combination of photoreduction and iron complexation (Boyd et al. 2000). Complexation by organic molecules known as ligands maintains dissolved iron concentrations by limiting its loss through scavenging and precipitation (Völker and Tagliabue 2015). We propose that iron released in whale faeces could potentially bind with organic ligands in seawater, or organic ligands could be released from whales along with the iron, but the magnitude of this complexation is uncertain, as reflected by our wide parameter ranges. Lastly, the consistency of whale faeces, being buoyant and liquid, could strongly influence the persistence in the photic zone (Smetacek 2008).

There is no information on the nature of dietary iron that is present in the gut of Antarctic krill. However Antarctic krill defecate faecal pellets enclosed within a membrane that could rapidly sink out of the photic zone, potentially limiting its uptake by phytoplankton. A field study

surveying 40 schools of krill over spring, summer and autumn in the Scotia Sea found that faecal pellet sinking rates varied between 27 – 1,218 m day^{-1} (median is 304 m day^{-1}), and this variability was mainly driven by pellet diameter and density (Atkinson et al. 2012). Based on the variable sinking rate and presence of a protective membrane, we assume that 10 – 30% of the iron defecated by Antarctic krill persists within the photic zone (p_k, Table 3.1). Of the faecal material that is retained, we assume that 10 – 50% of the defecated iron is bioavailable for phytoplankton uptake (ε_k, Table 3.1). Our poor understanding on the nature of krill faecal material (proportion of Fe^{2+}/Fe^{3+}) and the dissolution rates of the protective membrane limits the precise estimating of these two parameters.

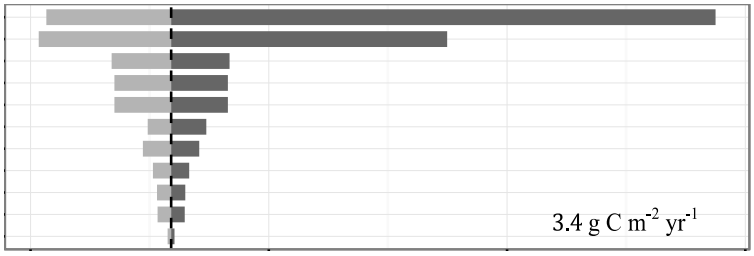
We estimated the whale and krill-induced primary productivity using the published range of molar carbon-to-iron ratio in phytoplankton from natural iron fertilisation studies and laboratory cultures (u, Table 3.1) (Twining and Baines 2004, Blain et al. 2007, Sarthou et al. 2008, Bowie et al. 2009). There is high variability in carbon-to-iron ratios due to species composition, growth rates, and iron bioavailability, amongst other controls, as well as methodological differences of quantifying iron supply (Twining and Baines 2004, Blain et al. 2007, de Baar et al. 2008, Sarthou et al. 2008, Bowie et al. 2009).

3.4 Results

Using the mean parameter values, the pre-exploitation population of blue whales and Antarctic krill, could have enhanced mean primary productivity by 0.3 g C m^{-2} yr^{-1} (Figure 3.2). At a minimum (PP_{min}), pre-exploitation blue whale populations and Antarctic krill enhanced primary productivity by 1.5 x 10^{-4} g C m^{-2} yr^{-1}, whilst at a maximum (PP_{max}), primary productivity could have been increased by 23.4 g C m^{-2} yr^{-1} (Figure 3.2). By comparison, Lavery et al. (2014), using a different method, estimate that historical populations of blue whales would stimulate primary productivity in the Southern Ocean by 1.3 x 10^{11} kg C yr^{-1} (or 6.5 g C m^{-2} yr^{-1} south of 60°).

Table 3.1 Estimates from the literature used to parameterise the model.

Symbol	Parameter	Blue whales			Fin whales			Humpback whales			Source
		Base	Minimum	Maximum	Base	Minimum	Maximum	Base	Minimum	Maximum	
N	Pre-exploitation population estimates	250,000	200,000	300,000	280,000	235,000	325,000	87,500	75,000	100,000	Branch et al. 2004; International Whaling Commission 2014, Leaper and Miller 2011
d	Feeding duration (days)	120	100	150	120	100	150	120	100	150	Lockyer 1981
α	Conversion factor	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	Ikeda and Dixon 1982
c	Daily consumption rate (kg day^{-1})	2917	1682	4130	1664	1309	2258	785	694	874	Lockyer 1981; Reilly et al. 2004
k	Concentration of iron in krill tissue (g kg^{-1})	0.0391	0.0044	0.1905	0.0391	0.0044	0.1905	0.0391	0.0044	0.1905	Barbante et al. 2000; Caroli et al. 1998; Deheyn et al. 2005; Kim et al. 2014; Locarnini and Preley et al. 1995; Nicol et al., 2010; Palmer et al. 2006; Ratnarajah unpublished data
f_w	Proportion of iron defecated by whales	0.8	0.7	0.9	0.8	0.7	0.9	0.8	0.7	0.9	Candela et al. 1984
f_k	Proportion of iron defecated by krill	0.8	0.7	0.9	0.8	0.7	0.9	0.8	0.7	0.9	*Assumption*
p_w	Proportion of whale faeces persisting in the photic zone	0.5	0.25	0.75	0.5	0.25	0.75	0.5	0.25	0.75	*Assumption*
p_k	Proportion of krill faeces persisting in the photic zone	0.2	0.1	0.3	0.2	0.1	0.3	0.2	0.1	0.3	*Assumption*
ε_w	Proportion of bioavailable iron from whales	0.5	0.25	0.75	0.5	0.25	0.75	0.5	0.25	0.75	*Assumption*
ε_k	Proportion of bioavailable iron from krill (%)	0.3	0.1	0.5	0.3	0.1	0.5	0.3	0.1	0.5	*Assumption*
u	Carbon: Iron in phytoplankton (mol mol^{-1})	140599	8264	416667	140599	8264	416667	140599	8264	416667	Blain et al. 2007; Bowie et al. 2009; Sarthou et al. 2008; Twinning et al. 2004



PP_{min} 1.4 x 10^{-4} g C m^{-2} yr^{-1}

PP_{max} 13.9 g C m^{-2} yr^{-1}

PP_{min} 2.4 x 10^{-5} g C m^{-2} yr^{-1}

PP_{max} 1.7 g C m^{-2} yr^{-1}

Figure 3.2 Influence of 11 model parameters on the estimate of whale and krill contributions to primary productivity in the Southern Ocean (south of 60°). We specifically considered the contribution of pre-exploitation levels of (top) blue, (centre) fin and (bottom) humpback whales and the biomass of krill required to support each population. Dashed vertical lines indicate primary productivity estimates from mean parameter values. *k* is the concentration of iron in krill tissue (in mg kg^{-1}), *c* is the daily consumption rate (in kg day^{-1}), *d* is the feeding duration (in days), *N* is the pre-exploitation population estimates, f_w and $f_k$is the proportion of iron defecated by whales and Antarctic krill respectively, p_w and p_k is the persistence of defecated iron by

whales and Antarctic krill respectively, ε_w and ε_k is the bioavailability of faecal iron by whales and Antarctic krill respectively, and u is the carbon: iron ratio in phytoplankton (in mol mol^{-1}). *w* and *k* subscripts next to parameters f, p and ε stand for whales and krill respectively. Note that the order of parameters on the y-axis changes between panels (i.e. between species of whale).

The flux of iron for the maximum estimate of primary productivity (PP_{max}) stimulated by blue whales and krill can be calculated from equation 5 by excluding the multiplication by u (the carbon-to-iron ratio, equal to 416667 mol mol^{-1} for the PP_{max} case, Table 3.1). This flux is equal to 0.03 μmol m^{-2} day^{-1} (Table 3.2), which is comparable to current estimates from the entire biomass of Antarctic krill (Table 3.2, Schmidt et al. 2011), the reported ranges for atmospheric deposition (Table 3.2, Bowie et al 2015, Duce and Tindale 1991, Fung et al. 2000), and upwelling (Table 3.2, Watson et al. 2001).

The contribution of the un-exploited fin whale population to primary productivity is lower than for blue whales, at 0.19 g C m^{-2} yr^{-1}, with a range of 1.4 x 10^{-4} (PP_{min}) to 13.9 (PP_{max}) g C m^{-2} yr^{-1} and the contribution of humpback whale populations is lower still, at 0.03 g C m^{-2} yr^{-1}, with a range of 2.4 x 10^{-5} (PP_{min}) to 1.7 (PP_{max}) g C m^{-2} yr^{-1} (Figure 3.2). To put these into perspective, current estimates of primary production in the Southern Ocean from remotely sensed ocean colour are in the order of 57 g C m^{-2} yr^{-1} (south of 50°, Arrigo et al. 2008).

Table 3.2 Dissolved iron flux (μmol m^{-2} day^{-1}) based on estimates from our preliminary model and published values for the Southern Ocean.

Location	**Source**	**Flux (μmol m^{-2} day^{-1})**	**Reference**
Southern Ocean	Blue whales and krill	0.03	This study
Scotia Sea	Antarctic krill	0.006 – 0.076	Schmidt et al. 2011
East Antarctica	Atmospheric dust	0.0016	Lannuzel et al. 2007
Southern Ocean	Atmospheric dust	0.005 – 0.05	Duce and Tindale et al. 1991
Southern Ocean	Atmospheric dust	0.0027 – 0.027	Fung et al. 2000
SOIREE patch	Atmospheric dust	0.00027	Bowie et al. 2001
Southern Ocean	Atmospheric dust	0.00055	Lefèvre and Watson 1999
Kerguelen region	Atmospheric dust	0.05 ± 0.039	Bowie et al. 2015
East Antarctica	Sea ice	0.3	Lannuzel et al. 2007
Kerguelen plateau	Diffusion	0.042 – 0.093	Bowie et al. 2015
Kerguelen plume	Diffusion	0.0005 - 0.001	Bowie et al. 2015
Weddell Sea	Icebergs	0.1 – 1	Shaw et al. 2011
East Antarctica	Vertical diffusion	0.01	Lannuzel et al. 2007
SOIREE patch	Vertical diffusion	0.003	Bowie et al. 2001
East Antarctica	Upwelling	0.12	Lannuzel et al. 2007
South of Polar Front	Upwelling	0.02 – 0.04	Watson 2001
Southern Ocean	Upwelling	0.13	de Baar et al. 1995
Kerguelen plateau	Upwelling	0.2 – 0.25	Bowie et al. 2015
Kerguelen plume	Upwelling	0.14 – 0.33	Bowie et al. 2015

3.5 Discussion

3.5.1 Overall contribution to primary productivity

Historical population levels of blue, fin and humpback whales may have enhanced primary productivity in the Southern Ocean (south of 60°). There is, however, a high degree of uncertainty around the magnitude of this enhancement due to uncertainty in model parameter estimates (Figure 3.2), and a significant role can only be observed when all parameters are at their upper limits. The fact that stimulated flux under the PP_{max} scenario for blue whales is equivalent in magnitude to several current fluxes suggests that this scenario may have required additional iron inputs to the system (particularly given that our model only captures one component of the ecosystem).

The ratio of mean primary productivity estimates due to iron recycling by all three species of whales and Antarctic krill to the amount of photosynthetic carbon consumed by the krill population required to support whale populations is 1.9 (i.e the χ ratio; Equation 6). This indicates that iron recycling by whales and krill is self-sustaining.

Although our overall estimate of the contribution of historical population levels of whales to primary productivity is relatively small when averaged across the entire Southern Ocean, iron recycling by whales and krill realistically impacts primary productivity in the ocean at local scales (e.g. in feeding areas). Consequently, the primary productivity induced by whale and Antarctic krill would be higher at a local scale as compared with our spatially averaged estimates.

3.5.2 Sensitivity to parameter uncertainty and guidance for future research

3.5.3 Influential parameters

For all three species of whales, the same parameters have the most influence on overall primary productivity estimates; however the order of influence on model output for certain parameters differed between the species. The top five parameters influencing the model were: the iron content in krill tissue (*k*); carbon-to-iron uptake ratio by phytoplankton (u); daily krill

consumption rates by whales (c) (except for humpback whales); and, the persistence (p_w) and bioavailability (ε_w) of the defecated iron by whales (Figure 3.2). For humpback whales, the feeding duration (d) was one of the top five most influential parameters (Figure 3.2).

In the literature, values of iron content in krill range over two orders of magnitude (Table 3.1). Observed variations of iron content in krill tissue could reflect seasonal and regional differences in the gut content of krill, the local presence of high iron source (e.g. hydrothermal vents), and could also be biased through sampling of different life stages, sample contamination or differences in analytical techniques (Deheyn et al. 2005, Schmidt et al. 2011, Schmidt et al. 2014). Antarctic krill primarily feed on phytoplankton in the spring and summer (Price et al. 1988). Over winter, they could be more omnivorous (Genhai 1993), feed on ice algae (Stretch et al. 1988) or tolerate starvation (Quentin and Ross 1991), all of which could affect their iron concentration. Furthermore proximity to submarine hydrothermal vents has been shown to also increase trace metal content in whole Antarctic krill in Bransfield Strait and Deception Island compared to areas with no hydrothermal influence (Deheyn et al. 2005, Tovar-Sanchez et al. 2009).

Crustaceans can also take up trace elements from the seawater into their body through their gills (Marsden and Rainbow 2004) and accumulate them within their tissues (Caroli et al. 1998). The accumulation of trace elements within a crustacean depends on the rates of uptake from seawater and diet, excretion rate of the trace element and the growth rate of the crustacean (Marsden and Rainbow 2004). The continuous accumulation of trace metals, however, would be toxic. Consequently, to avoid potential toxic effects, these trace elements must be detoxified or excreted (Rainbow 2002). Because krill can take up iron from the phytoplankton they ingest, and directly from the water, they may play a role in recycling iron as well as sequestering iron making it unavailable for primary producers.

The wide range in the carbon-to-iron cellular ratio in phytoplankton greatly influenced primary productivity estimates and this is largely due to: specific requirements of different species of

phytoplankton (Twining et al. 2004, Twining and Baines 2004), individual growth rate (Coale et al. 1996, Timmermans et al. 2001b, Timmermans et al. 2004), size (Gall et al. 2001), surface area to volume ratio (Timmermans et al. 2001a, Timmermans et al. 2001b, Timmermans et al. 2004), and ambient concentrations of iron. Phytoplankton growing in iron-replete waters have lower carbon-to-iron ratios (Sunda and Huntsman 1997, Twining and Baines 2004).

The relative importance of consumption rate varied between the three species of whales. There are no direct measurements of the daily krill consumption rates by baleen whales. However, a variety of methods have been used to obtain estimates of prey consumption that include using daily prey consumption from average body weight (35g kg^{-1} body weight), and 2%, 2.5% and 3% of body mass (Lockyer 1981, Reilly et al. 2004). Consumption rates, which directly influence the amount of iron being consumed and defecated, are indeed likely to vary across species, life stages, as well as with prey abundance.

No published measurement of the persistence of faecal material in the photic zone is available. In our model, we assume retention rates of between 25 – 75%, and the bioavailable fraction of this retained iron also between 25 – 75%. These ranges were chosen due to the lack of information in the size fractionation of faecal material (i.e. the concentration of iron that is associated with the particulate and dissolved pool), the fraction of particulate iron that can leach and remain in solution, concentration of iron-binding ligands and the effects of varying environmental conditions on ligand production. Organic complexation is a key factor in ocean iron cycling, controlling iron solubility (Wu and Luther 1995) and bioavailability (Maldonado et al. 2005, Hassler and Schoemann 2009). Complexation of iron by organic ligands could increase the residence time of dissolved iron in surface waters (Hunter and Boyd 2007) and potentially enhance iron uptake by phytoplankton; however these interactions are not fully understood at present.

In vertebrates, most of the dietary iron entering the intestine is in the oxidised ferric form, but the hydrochloric acid and pepsin in the stomach, and the low oxygen environment in the intestine

would favour the reduction of iron to ferrous iron (Fe^{2+}) (Slijper 1962, Naikare et al. 2006). As the faecal material is expelled into an oxygenated environment, the ferrous iron could return to an oxidised ferric form. The oxidised ferric iron could bind to organic ligands to form iron-organic ligand complexes and retain iron in solution when faecal iron dissolves in seawater (Hunter and Boyd 2007). The concentration of iron complexing ligands in Southern Ocean waters is approximately 0.72 ± 0.23 nM L^{-1} (Boye et al. 2001), therefore, Southern Ocean seawater may not contain sufficient organic ligands to bind with a large pulse of faecal iron. Some of the whale faeces could already be released in the dissolved form bound to ligands in the organic-rich material, however this has yet to be demonstrated.

3.5.4 Least influential parameters

Better constrained parameters and those that had relatively less influence on model estimates of the contribution of whales to primary productivity were: feeding duration in polar waters (d) (except for humpback whales), whale population size estimates (N), proportion of dietary iron defecated by whales (f_w) and Antarctic krill (f_k) and the persistence (p_k) and bioavailability (ε_k) of Antarctic krill faecal pellets. Baleen whales undergo extensive migrations to Antarctic waters over summer and northward for the winter; however migration times may vary with life stages (Lockyer 1981). Peak densities in Antarctic waters occur between December to February with immature whales reaching peak abundance later in the season than mature whales (Lockyer 1981).

The range of estimates for the historical population sizes of blue, fin and humpback whales has a lower influence on productivity estimates than other parameter discussed above (Figure 3.2). Prior to commercial exploitation, large baleen whales were extremely abundant. Antarctic blue whales are currently thought to be amongst the most endangered of the great whales (Clapham et al. 1999, Branch et al. 2004), and they have been protected worldwide since the 1960's (International Whaling Commission 2014).

The best estimate of the current population size of Southern Hemisphere blue whales is approximately 2,300 individuals (95% confidence limit (860 – 4,500 individuals) between 1996 and 1998 (International Whaling Commission 2014). Using a mean estimate of 2,300 individuals, our model predicts that primary productivity stimulated by blue whales and the biomass of Antarctic krill supporting them in the Southern Ocean would only be 2.7×10^{-3} g C m^{-2} yr^{-1} (minimum of 6.6×10^{-7} g C m^{-2} yr^{-1} from 860 individuals, and a maximum of 0.35 g C m^{-2} yr^{-1} from 4,500 individuals). These mean primary productivity estimates are approximately 100 times less than estimates using the pre-exploitation population size.

The population recovery rate for some populations of humpback whales in the Southern Hemisphere is greater than those of blue and fin whales. Current estimated Southern Hemisphere humpback whale abundance is around 42,000 individuals (95% confidence limit of 34,000 – 52,000 individuals between 1997 and 1998) (International Whaling Commission 2014). Our model predicts that the current population of humpback whales could contribute to an enhancement in primary productivity of 1.3×10^{-2} g C m^{-2} yr^{-1} (minimum of 1×10^{-5} g C m^{-2} yr^{-1} from 34,000 individuals, and a maximum of 0.9 g C m^{-2} yr^{-1} from 52,000 individuals). There are no reliable data on the current status of the Southern Hemisphere fin whale populations. Here we only consider historical and present estimates. Future studies could usefully consider primary productivity estimates over intermediate whale population sizes.

The proportion of iron consumed and subsequently defecated by whales is likely to vary with life stage (young vs. mature) and sexual maturity (male and female vs. lactating and pregnant females). Young whales require more iron to build muscle. Consequently, they likely defecate less iron than adult whales, and would have a lesser effect on primary productivity – however this has not been quantified. Adult mammals most likely defecate most of the dietary iron consumed, unless they are injured, pregnant or lactating in which case they will be storing some of the dietary iron for growth and milk production. Lactating females also excrete iron through their milk. The composition of milk from the stejneger's beaked whale (*Mesoplodon stejnegeri*) was found to contain 35 mg kg^{-1} of iron (Ullrey et al. 1984). In a rapidly growing population,

there would be a much higher proportion of pregnant or growing individuals than in the pre-exploitation “stable” population. Additional information on the iron content in the muscle of adult and calf whales would be useful in further understanding the effects of whales on primary productivity in the Southern Ocean, but these measurements would be less critical than the other measurements highlighted above (3.5.3 Influential parameters).

The proportion of iron defecated and the persistence and bioavailability of faecal material by Antarctic krill had little influence on model output. The low importance of the persistence and bioavailability of faecal iron could have been because of the constrained ranges used in the model. These constrained ranges were chosen due to the consistency of Antarctic krill faecal material that is in the form of a faecal pellet enclosed within a membrane, which could rapidly sink out of the photic zone. This is in contrast to the fluid and buoyant nature of whale faecal material. Additional investigations on these parameters would better guide subsequent models to compare potential iron contributions of Antarctic krill with that from whales.

3.5.5 Caveats and limitations

The model presented here is preliminary and helps prioritise key areas for future research. As such, there are some caveats to the interpretation of this analysis that could be resolved in a dynamic model as more information becomes available. First, our model assumes a linear relationship between primary productivity and iron re-supply by whales and krill. The model does not capture the actual limitation of photosynthesis by iron, and our results do not account for the potential change of status of the Southern Ocean waters from iron-limited to iron-replete for phytoplankton production. When the iron supply exceeds consumption by phytoplankton, then dissolved iron could accumulate in surface waters and iron may no longer be limiting, as evidenced where iron is supplied through dust deposition (Guieu et al. 2002, Sedwick et al. 2005), coastal upwelling (Johnson et al. 1999), river plumes (Lohan and Bruland 2006), seasonal ice zones (Sedwick and Di Tullio 1997), and islands (Bucciarelli et al. 2001).

Our model does not include limitations of whale and krill growth due to food availability. We assume that the biological recycling of iron by whales could increase phytoplankton production rates, which in turn would support a larger Antarctic krill biomass (Smetacek 2008). In addition, our analysis only partially captures the cycling of iron (Figure 3.1), and assumes that some iron flows in and out of our model system.

The mode of iron supply and uptake by phytoplankton is also not represented in our static model. In iron fertilisation experiments, iron supply (episodic or continuous) differentially influences the diversity and growth of phytoplankton. *In-situ* artificial iron fertilisation experiments often result in a shift from natural phytoplankton assemblages to dominance by large diatoms (Boyd et al. 2000, see review in Boyd et al 2007), or result in mixed assemblages (Blain et al. 2007). Continuous chemostat addition of iron instead results in a phytoplankton community co-dominated by both small diatoms and nanophytoplankton (Hare et al. 2007). In addition, some phytoplankton experience luxury iron uptake, where they maintain high iron uptake rates despite already achieving maximum growth rates, allowing them to better adapt to episodic iron supply (Sunda and Huntsman 1995, Kustka et al. 2003).

Our model assumes that iron is the main limiting nutrient in the Southern Ocean; however light limitation due to deep vertical mixing has also been demonstrated to affect primary productivity (de Baar et al. 2005). A greater amount of iron is required to support phytoplankton growth under low light intensities (Sunda and Huntsman 1997). This is because phytoplankton acclimated to low light conditions have a greater requirement for photosynthetic iron-based redox proteins (Sunda and Huntsman 1997). Furthermore, iron uptake by phytoplankton varies with cell surface areas, where smaller cells are favoured under iron limiting conditions (Hudson and Morel 1990, Sunda and Huntsman 1997, Timmermans et al. 2001b).

Silicate concentration in the Sub Antarctic zone is also low (<1.1 μmol L^{-1} in Lannuzel et al. 2011 and Bowie et al. 2009), which could co-limit phytoplankton growth (Hutchins et al. 2001). Under nutrient-replete conditions, diatoms use silicic acid and nitrate under normal molar ratios

of approximately 1: 1 (Sarthou et al. 2005). Under iron-limited conditions, diatoms use silicic acid and nitrate at molar ratios of 2: 1 or higher (Hutchins and Bruland 1998, Coale et al. 2004). Therefore in low iron regions, the heavy utilization of silicic acid would lead to co-limitation by both iron and silicate (Sedwick et al. 2002). Silicate limitation is also not considered in our model.

Our model is not spatially explicit. Iron concentration in surface seawater is higher where it is supplied by shelf sediments (Sedwick et al. 2008, Bowie et al. 2009), melting sea ice (Sedwick and Di Tullio 1997, Lannuzel et al. 2007) and icebergs (Smith et al. 2007, Lin et al. 2011), and through internal mixing processes (Tagliabue et al. 2014). Here we assume that iron-rich defecation by whales would have an equal effect where these other sources are co-located. A spatially and seasonally resolved model would be the next step to accurately capture patterns of iron contribution by whales and Antarctic krill to phytoplankton growth. Lastly, we intentionally do not extrapolate potential contribution of iron through biological recycling on carbon export due to uncertainties in the persistence and bioavailability of faecal iron in the photic zone, phytoplankton species specific carbon-to-iron ratios, uncertainties in estimating carbon cycling through the food web, and sinking rates of un-grazed phytoplankton.

3.6 Conclusion

This model attempts to determine the potential contribution of historical populations of whales and krill to iron fertilisation in the Southern Ocean, and quantify uncertainty due to our limited understanding of key biogeochemical processes. In order to refine these estimates of the role of current and historical whale contribution to recycling of iron, future research should focus on quantifying: (i) the iron content of krill, (ii) the krill consumption rate by whales, (iii) the persistence of faecal iron in the photic zone, (iv) the bioavailability of this retained iron, and (v) the carbon-to-iron ratio of Southern Ocean phytoplankton.

3.7 Acknowledgements

We would like to thank Tino Bowie-Proemse for insightful discussions, Prof. Tom Trull, Dr. So Kawaguchi, Prof. Philip Boyd, Dr Mike Double, Dr Bill de la Mare, and the two anonymous reviewers for their comments and suggestions during the development of this project and manuscript. This study was supported by the Australian Government's Cooperative Research Centres Programme through the Antarctic Climate and Ecosystem Cooperative Research Centre, the Institute for Marine and Antarctic Studies, the Holsworth Wildlife Research Endowment, the European Union COST Action ES0801 and through Australian Antarctic Science grants 4343 and 4347.

4 Understanding the variability in the iron concentration of Antarctic krill

This chapter has been published:
Ratnarajah L, Nicol S, Kawaguchi S, Townsend AT, Lannuzel D, Meiners KM, Bowie AR. (2016b) Understanding the variability in the iron concentration of Antarctic krill. Limnology and Oceanography 61:1651 – 1660 doi: 10.1002/lno.10322

4.1 Abstract

Antarctic krill may play a significant role in the Southern Ocean iron cycle. However understanding the control on iron budgets by Antarctic krill is hampered by the large range in the reported iron concentration of krill. The aim of this study was to investigate the causes of the large range of iron concentrations in krill reported in the literature (6 – 190 mg kg^{-1}). Antarctic krill samples were collected from three research voyages to Pyrdz Bay, Antarctica, and analysed individually. Iron concentrations were measured using sector field inductively coupled plasma mass spectrometry in whole krill specimens and in the isolated stomach, digestive gland, muscle, body (whole krill excluding stomach and digestive gland), exoskeleton and faecal pellets.

Iron concentrations in stomach (6 – 98 mg kg^{-1}), digestive gland (14 – 82 mg kg^{-1}) and faecal pellet (683 – 1039 mg kg^{-1}) were higher compared to muscle (4 – 7 mg kg^{-1}), exoskeleton (6 – 15 mg kg^{-1}) and body (4 - 18 mg kg^{-1}) indicating that krill may ingest more iron than they require for physiological processes. Iron concentrations in whole krill from March 2012 (10 ± 3 mg kg^{-1}) were significantly lower compared to February 2003 (19 ± 7 mg kg^{-1}) and February 2015 (18 ± 12 mg kg^{-1}). Overall, the iron concentrations in krill from this study were consistently at the lower end of the published range. We propose that the large range in reported whole iron concentrations of krill can be accounted for by a combination of seasonal and regional differences in sampling, reflecting differences in the quantity and quality of their diet.

4.2 Introduction

Antarctic krill (*Euphausia superba*, hereafter referred to as krill) is a keystone species in the Southern Ocean ecosystem. They exhibit a circumpolar distribution south of the Polar Front, with approximately 70% of their stock present in the Atlantic sector (Atkinson et al. 2008). With a biomass of between 100 to 500 million tonnes (Atkinson et al. 2009) found mostly in the upper 150 m of the water column (Demer and Hewitt 1995, Lascara et al. 1999), krill are both a major grazer of primary productivity in the Southern Ocean, and major prey for many marine vertebrate predators (Everson 2000, Pakhomov et al. 2002).

Recent studies have demonstrated that in the Southern Ocean, krill could play an important role in storing iron (Fe) obtained through their diet (Nicol et al. 2010, Ratnarajah et al. 2014). Lower trophic level crustaceans store Fe in their body muscle and cuticle for physiological requirements (Marsden and Rainbow 2004, Nicol et al. 2010, Ratnarajah et al. 2014). Nicol et al. (2010) estimated that the Southern Ocean krill population could contain approximately 24% of the total Fe in the surface waters in the region, acting as a Fe reservoir. Iron not required for physiological processes by krill is either defecated or, if absorbed, can be excreted through their antennal glands, gills and guts (Marsden and Rainbow 2004, Tovar-Sanchez et al. 2007, Schmidt et al. 2011, Lehette et al. 2012).

Only four studies have explicitly examined the role of krill in storing and recycling Fe in Southern Ocean surface waters (Tovar-Sanchez et al. 2007, Nicol et al. 2010, Schmidt et al. 2011, Ratnarajah et al. 2014). Other studies have examined Fe concentration in krill as a means to understand marine pollution (Locarnini and Presley 1995, Palmer et al. 2006), as a candidate reference material (Caroli et al. 1998, Barbante et al. 2000), nutritional composition (Kim et al. 2014), or studying the influence of hydrothermal vents on biochemical composition (Deheyn et al. 2005, Tovar-Sanchez et al. 2009). Iron concentrations in whole krill reported in these studies are far higher than background seawater concentrations, consistent with the suggestion that krill are a reservoir of Fe in Southern Ocean surface waters. However, there is high variability within the reported values of Fe concentrations in whole krill (6 – 190 mg kg^{-1}), which strongly

influences our understanding of the role of krill in the Southern Ocean Fe cycle (as an Fe store) and the recycling of Fe by higher trophic levels (e.g. krill-feeding baleen whales) in the Southern Ocean (Ratnarajah et al. 2016a).

Iron limits primary productivity in large areas of the Southern Ocean. The Fe concentrated in krill is transferred to higher order marine animals when they are eaten, and ultimately recycled through the defecation of excess Fe into Fe-poor surface waters of the Southern Ocean (Nicol et al. 2010, Ratnarajah et al. 2014). Adult whales, like all mammals, do not require all the Fe that they ingest and, because they cannot excrete iron once it has been absorbed, most of it is defecated (Smetacek 2008, Nicol et al. 2010, Ratnarajah et al. 2014). Baleen whales take in Fe that is unavailable for phytoplankton growth (because it is locked up in the bodies of krill) and convert it to a faecal slurry that could be a stimulant for phytoplankton growth (Smith et al. 2013).

Calculations have suggested that pre-exploitation populations of blue whales (*Baleoptera musculus*) could have resulted in a contribution to primary productivity of 23.4 g C m^{-2} yr^{-1}, when all parameter estimates are at their upper limits, including the Fe concentration in krill (190 mg kg^{-1}, in Kim et al. 2014), but only 1.5 x 10^{-4} g C m^{-2} yr^{-1} when all parameter estimates are at their lower limits, including the Fe concentration in krill (4 mg kg^{-1}, from this study) (Ratnarajah et al. 2016a). In this preliminary model, the variability of Fe concentration in krill was consistently the most influential parameter determining the potential of blue, fin (*B. physalus*) and humpback (*Megaptera novaeangliae*) whales to affect productivity in the Southern Ocean. It is important to understand what drives this large range in Fe concentrations, as it forms the base for a greater understanding of the efficiency of biological recycling by higher order marine animals in the Southern Ocean.

The aim of this study was to understand and constrain the range of reported values for Fe concentration in whole Antarctic krill. In addition to the Fe concentration in whole krill, the concentration of Fe in krill stomach, digestive gland, muscle (from the abdominal segments,

without exoskeleton), body (only excluding stomach and digestive gland), exoskeleton and faecal material were examined. The concentration of Fe in the stomach reflects what has recently been consumed and could be assimilated and/or recycled. The digestive gland serves the dual role of secreting enzymes and absorbing digested food and reflects what food is in the process of being utilised. The muscle and body reflects what is assimilated, the exoskeleton reflects what could be absorbed through the diet or adsorbed from seawater, while the faecal material reflects excess Fe that is in the process of being defecated. The carbon (C) content and Fe/C ratio of whole krill, stomach, digestive gland and body (only excluding stomach and digestive gland) were also determined. The Fe/C ratios between each body part are used as a proxy to trace the metabolic pathway of Fe trough the krill to determine if the Fe consumed is being stored or recycled.

4.3 Methods

4.3.1 Sample collection

Krill samples were collected using a Rectangular Midwater Trawl net (RMT 8) on the *Aurora Australis* on three research voyages in Prydz Bay, Antarctica (1 February 2003, 5 March 2012, and 22 February 2015). Whole krill were individually snap frozen by liquid nitrogen in cryotubes immediately after their capture and stored frozen at -20°C until analysed. As krill tend to defecate when caught, 300 freshly caught krill (22 February 2015) were placed into three plastic buckets (100 specimens in each) to collect faecal pellets. Faecal pellets were removed using disposable plastic pipettes and placed into three vials (one for each bucket) and frozen at -20°C until analysed.

4.3.2 Analysis of iron

Low-density polyethylene (LDPE) nutrient tubes (10 mL) were used for drying krill specimens, sample dilution, and analysis, and polymethylpentene (PMP) forceps were used for dissection. New LDPE nutrient tubes and PMP forceps were soaked in 2% Decon 90 (Decon Laboratories) cleaning solution for at least 7 days and acid leached in 10% (v/v) Hydrochloric acid (HCl, Merck, Analytical grade 32%, Germany) for 4 weeks prior to use. Teflon perfluoroalkoxy (PFA)

screw cap digestion vials (15 mL; Savillex Corp., USA) were acid leached in 50% (v/v) HCl for 2 weeks. Following acid leaches, all materials were rinsed thoroughly (five times) with Ultra High Purity (UHP) water and left to dry in an HEPA filtered Class 100 laminar flow bench.

Krill specimens collected on each voyage were dissected in the laboratory with acid washed PMP forceps to obtain stomach, digestive gland (also commonly known as the midgut gland or hepatopancreas), and body samples. The exoskeleton and abdominal muscle samples (without exoskeleton) were dissected using sterile, disposable scalpels with stainless steel blades. Scalpels were rinsed with UHP water prior to use and discarded after each use. The low Fe concentrations measured for these samples indicated that the stainless steel scalpels had no contamination effect. Some krill specimens from each voyage were not dissected and were analysed whole.

All samples were dried at 60°C in acid-cleaned LDPE tubes for 48 hours to constant weight. Digestion of 3 – 403 mg (± 0.1 mg) subsamples were performed in acid-cleaned 15 mL Teflon® PFA vials by adding 1 mL of concentrated nitric acid (HNO_3) and 0.125 mL of hydrogen peroxide (H_2O_2) (all Ultrapure, Seastar Baseline®, Choice Analytical). Samples were then heated at 125°C for 8 hours on a Teflon coated digestion hotplate, housed in a bench-top fume hood coupled with HEPA filters to ensure clean air input (Digiprep, France). Additional samples collected on 22 February 2015 were also digested with hydrofluoric acid (HF) to compare the efficiency between digestions with and without HF present. The addition of HF allows for greater digestion of refractory elements compared to the HNO_3 and H_2O_2 assisted digestion (see Section 4.1.2). For these samples, 0.5 mL of HF (Ultrapure, Seastar Baseline®, Choice Analytical) was added to 1 mL HNO_3 and digested following the same procedure. Samples were then allowed to cool overnight to prevent handling hot acids.

Following digestion all samples were dry evaporated at 60°C for 4 hours on the Teflon coated digestion hotplate, cooled for ~2 hours, then resuspended in 10 mL of 10% v:v HNO_3 (Ultrapure, Seastar Baseline). Identical procedures were applied to digest blanks (i.e. HNO_3 and H_2O_2, n = 14 and HNO_3 and HF, n = 3), and to three certified referenced materials (CRMs) (DORM-3 fish

protein, MESS-3 marine sediment, and BCR-414 plankton) to assess elemental recovery following digestion.

Prior to analysis all samples, CRMs and digest blanks were diluted 100-fold in 2% v:v HNO_3 (Ultrapure, Seastar Baseline). Indium (In) (High-Purity Standards, USA) was added to all samples at a final concentration of 10 μg L^{-1} and used as an internal standard. Representative subsamples from each analytical sequence were also spiked with a multi-element solution (QCD Analysts, MISA suite of solutions, 10 μg L^{-1}, Spring Lake, USA) to monitor elemental recoveries in the sample matrices considered.

Analyses were conducted using a sector field inductively coupled plasma mass spectrometry (ICP-MS) (Thermo Fisher MAT ELEMENT 2 Bremen Germany), following methods described in Bowie et al. (2010) and Townsend (2000). This ICP-MS has three pre-defined spectral resolutions available enabling isotopes to be quantified with minimal spectral interferences. Iron was measured using the "medium" resolution setting (m/dm ~ 4000). Four calibration standards with concentrations 0, 1, 5 and 10 μg L^{-1} were prepared by serial dilution from multi-element stock solutions (QCD Analysts, MISA suite of solutions, Spring Lake, USA). Samples were generally organised for analysis from lowest to highest concentration (muscle to organs) to minimise sample-sample carry-over and associated instrumental memory effects (Bowie et al. 2010).

4.3.3 Analysis of carbon

Whole krill, krill body (only excluding stomach and digestive gland), stomach and digestive gland from 10 individuals collected in February 2003 were dried at 60°C and homogenised using an acid cleaned agate pestle and mortar. Six subsamples from each homogenised body part, and faecal material, were placed in individual 8 x 5 mm tin capsules (Elemental Microanalysis, UK) and weighed (approximate range 0.7 – 1.7 mg). Carbon content was determined using a Thermo Finnigan EA 1112 Series Flash Elemental Analyser (estimated precision ~1%) at the Central Science Laboratory, University of Tasmania.

4.3.4 Statistical analysis

Type II ANOVA was used for unbalanced data to determine if there were significant differences in whole krill between the three sampling dates, and between the two digestion methods (Langsrud 2003). The Fe concentrations were log-transformed to meet the assumptions of ANOVA. Tukey HSD post hoc test was then conducted to examine the significance of differences between voyages. We did not test for significant difference in means of the stomach and digestive gland between voyages because of the low mass and small sample size for these organs. Statistical analysis was performed using R (R Core Team 2014).

4.4 Results

Good agreement between measured and certified values were achieved for Fe in the CRMs (Table 4.1), with recoveries averaging 79% for DORM-3, 87% for BCR-414, and 72% for MESS-3, all using HNO_3 alone. The recovery for MESS-3 using a combination of HF and HNO_3 was 88%. These results confirm that the use of HF ensures a greater digestion (soluble and refractory) of the CRM sample. Average spike recovery for Fe near 95% was typically found for both digestion protocols, with and without the presence of HF. Average Fe blanks were 0.06 ± 0.02 μg L^{-1}, and theoretical detection limits (defined as three times the standard deviation of the blank) were 0.07 μg L^{-1}. Mean Fe concentrations determined for krill stomach, digestive gland, muscle, body, exoskeleton, whole krill and faecal material from this study are summarised in Table 4.2; and comparative values of Fe concentrations for Antarctic krill from the literature are presented in Table 4.3.

The three-way ANOVA demonstrated a significant difference in Fe concentrations in whole krill between the three sampling periods ($p < 0.01$). The Tukey multiple comparison of means indicated that the differences in determined Fe concentrations between whole krill collected in February 2003 and February 2015 were not significantly different ($p = 0.8$). However, the concentration of Fe in whole krill collected in March 2012 was significantly different from that collected in February 2003 ($p < 0.01$) and February 2015 ($p < 0.01$). There was no difference in

the mean Fe concentrations measured between whole krill digested in HNO_3 compared to samples digested with additional HF ($p = 0.2$, Figure 4.1).

Table 4.1 Iron concentrations determined using sector field inductively coupled plasma mass spectrometry (SF-ICP-MS) for certified referenced material (CRM) of fish protein (DORM-3), plankton (BCR-414) and marine sediment (MESS-3). Recovery values indicate the percentage difference between measured and indicative or certified values. Reference values for DORM-3 and MESS-3 are certified (results accepted for certification by the participating laboratories) and BCR-414 is indicative (elements determined individually by some laboratories). Values as mg kg^{-1}.

CRM	**Reference values**	**Measured average ± Standard deviation**	**Recovery (%)**
DORM-3 (n=6)	347	274 ± 19	79
BCR-414 (n=6)	[1,850]	1,615 ± 227	87
MESS-3 (n=3)*	43,400	31,071 ± 321	72
MESS-3 (n=3)**	43,400	38,273 ± 1460	88

Average BCR-414 value was obtained from measurements on 4 separate occasions (27 April 2012, 25 November 2014, 15 July 2015 and 15 August 2015). Average values for DORM-3 and MESS-3* were determined across 2 periods (27 April 2012 and 15 March 2015, and 23 June 2015 and 20 August 2015, respectively).

* MESS-3 with nitric acid and hydrogen peroxide digestion

** MESS-3 with nitric acid and hydrofluoric acid digestion

Table 4.2 Mean iron concentrations in Antarctic krill specimens from Prydz Bay, Antarctica (mg kg^{-1} dry weight).

Sample type	**Collection date**		
	Feb 2003	**Mar 2012**	**Feb 2015**
Whole krill	19 ± 7 (n = 14)	10 ± 3 (n = 15)	18 ± 12 (n = 20)
Stomach	18 ± 12 (n = 16)	39 ± 41 (n = 12)	34 ± 6 (n = 10)
Digestive gland	36 ± 23 (n = 16)	38 ± 16 (n= 13)	25 ± 1 (n = 15)
Muscle*	5 ± 1 (n = 4)	5 ± 1 (n = 4)	5 ± 1 (n = 6)
Krill body**	11 ± 4 (n = 19)	7 ± 2 (n = 20)	11 ± 3 (n = 18)
Exoskeleton	15 ± 3 (n = 6)	11 ± 3 (n=5)	14 ± 1 (n = 6)
Faecal material	n/a	n/a	861 ± 252$^{\delta}$

n/a No available sample

*Krill muscle from the 5th and 6th abdominal segment only, excluding exoskeleton

**Krill body excludes stomach and digestive gland only

$^{\delta}$ Sample size uncertain because not all krill would have defecated into the bucket

Table 4.3 Published average iron concentrations (mean ± standard deviation) in Antarctic krill from the Southern Ocean (mg kg^{-1} dry weight).

Sampling location	**Month of sampling**	**Method**	**Sample type**	**Fe**	**Reference**
Southern Ocean 66°04'27"S, 109°58'95"E	27 Mar 2007	ICP-MS	Whole krill	174 ± 0.5	Nicol et al. 2010; Ratnarajah et al. 2014
Scotia Sea	Jan – Feb 2002, 2003, Mar 2004	ICP-MS*	Muscle	3	Schmidt et al. 2011
			Stomach	2783	
Various locations in Antarctic waters	1993 – 1994, 1994 – 1995	ICP-MS	Whole	56 ± 6	Barbante et al. 2000
Southern Indian Ocean 66°30'33"S, 69°34'48"E	11 Feb 2003	ICP-MS	Whole	15 ± 1	Palmer et al. 2006
Western Antarctica Peninsula	Fall 1993	Flame AAS	Whole	9 - 108	Locarnini & Presley 1995
Uncertain	Mar – Aug	ICP-MS	Whole	34 – 190	Kim et al. 2014
Ross Sea	1993 – 1994	ICP-MS and ICP-AES	Whole	8 ± 0.02	Caroli et al. 1998
Marguerite Bay	1994 – 1995	ICP-MS and ICP-AES	Whole	6 ± 0.07	Caroli et al. 1998
Livingston Island	1994 – 1995	ICP-MS and ICP-AES	Whole	6 ± 0.02	Caroli et al. 1998
Deception Island, Antarctic Peninsular Port Foster mid-caldera	November 2000	ICP-AES	5^{th} segment	140 ± 20	Deheyn et al. 2005

ICP refers to Inductively Coupled Plasma, MS refers to Mass Spectrometry, AES refers to Atomic Emission Spectroscopy and Flame AAS refers to Flame Atomic Absorption Spectroscopy

ICP-MS* used hydrofluoric acid

Kim et al. 2014 demonstrated statistically significant monthly variation in Fe concentration in Antarctic krill

Caroli et al. 1998 in wet weight. Conversion factor of 0.23 from wet weight to dry weight (Nicol et al. 1992)

A large range in Fe concentration in the stomach, digestive gland and faecal material was observed (Table 4.2). Except for samples from February 2003, the mean Fe concentrations in the stomach were higher than the digestive gland, and both organs showed higher concentrations than were observed in the whole body or muscle (Table 4.2). Interestingly, the concentration of Fe in the muscle of krill (excluding the exoskeleton) was the same for all three sampling periods (Table 4.2). Highest Fe concentrations were found in the faecal material (Table 4.2).

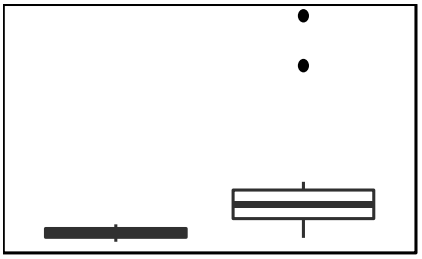


Figure 4.1 Comparison between nitric acid (HNO_3) and hydrogen peroxide (H_2O_2) with nitric acid (HNO_3) and hydroflouric acid (HF) digestion in whole krill, krill stomach, digestive gland and muscle samples from February 2015 (in mg kg^{-1}). Horizontal line within each plot represents the median.

To determine the Fe/C ratio of whole krill and their body parts, Fe and C measurements were performed on a homogenate of 10 individual samples, subsampled for both analyses. When normalised to C, the concentration of Fe was highest in the digestive gland (13.7 ± 1.6 μmol mol^{-1}) followed by stomach (7.1 ± 0.6 μmol mol^{-1}) and krill body (4.3 ± 0.6 μmol mol^{-1}) (Figure 4.2). The Fe:C ratio in whole krill was 10.3 ± 4.3 μmol mol^{-1} (Figure 4.2).

Figure 4.2 Iron to carbon ratio in digestive gland, stomach, body (only excluding stomach and digestive gland) and whole krill samples from February 2003 (μmol mol^{-1}). Horizontal line within each plot represents the median.

4.5 Discussion

4.5.1 Comparison to the literature

4.5.2 Iron concentrations

The Fe concentrations in whole krill from this study were within the reported ranges for Antarctic krill in Palmer et al. (2006), but were at the low end of the range reported in other published studies (Table 4.3). However, the mean total Fe concentration in whole krill from this study is still over 1 million times higher than observed in Antarctic open surface waters (~ 0.2 nmol L^{-1} or ~10 ng kg^{-1}; Bowie et al. 2001).

A significant difference in Fe concentrations was observed between the krill sampled in February 2012 and 2015 with krill sampled in March 2003. This may reflect interannual variability in seasonal timing, yearly variations in phytoplankton abundance, or the gradual decline in primary productivity from summer to winter (Moline and Prezelin 1996, Smith Jr et al. 2000, Marrari et al. 2008). The latter is consistent with findings from Kim et al. (2014), who demonstrated significant monthly variations in the nutritional composition of krill from March to August.

The concentration of Fe in whole krill from this study is lower than that observed in other Antarctic invertebrates: planktonic copepods (208 mg kg^{-1}), the Antarctic scallop (*Adamussion colbecki*, 230 – 880 mg kg^{-1}, n = 27) (Honda et al. 1987) and the amphipod *Paramoera walkeri* (98 – 165 mg kg^{-1}, n = 1) (Palmer et al 2006). Other species of krill exhibit both higher and lower iron concentrations than that reported here: *Meganyctiphanes norvegica* (12 ± 3 mg kg^{-1}, n = 10), *Pseudeuphausia latifrons* (151 mg kg^{-1}, n = 5), *Nyctiphanes australis* (91 mg kg^{-1}, n = 5), *E. pacifica* (62 mg kg^{-1}, n = 5) and *E. krohnii* (34 mg kg^{-1}, n = 5) (Nicol et al. 2010).

Only one other study has reported Fe concentrations in krill stomach samples. The Fe concentration in krill stomach measured by Schmidt et al. (2011) was approximately 1,000 times higher than our measured concentrations. However, both studies measured concentrations of Fe in the digestive organs that were much higher than in muscle (Table 4.2 and 4.3). This suggests that the variation noted may be caused by the concentration of Fe in the krill diet.

4.5.3 Methodological and analytical differences

Some of the variation in the reported values of Fe concentrations in krill could be a result of methodological and analytical differences. Most reported investigations in Table 4.3 used a HNO_3-assisted digestion (no HF present), however Schmidt et al. (2011) also employed HF (in addition to HNO_3 and hydrochloric acid, HCl) during the digestion step, added perchloric acid ($HClO_4$) and evaporated at 140°C and subsequently at 170°C. Hydrofluoric acid allows for greater digestion of the more refractory trace elements compared to HNO_3 (Bowie et al. 2010).

This is consistent with the recoveries determined in this work for CRM's (Table 4.1). Using only HNO_3, good recoveries for Fe in BCR-414 (phytoplankton, 90%) and DORM-3 (fish protein, 79%) were noted, both of which are mostly soluble material (Table 4.1). However, for MESS-3 (marine sediment), HNO_3 digestion only recovered approximately 70% of the total Fe, whilst with HF added, the digestion recovery was 88 % of the total Fe (Table 4.1). Measured CRM data suggest that HNO_3 only digestion is only suitable for the recovery of labile particulate Fe, and some portion of the refractory fraction. All other studies reported in Table 4.3 used a HNO_3 only digestion.

There are also differences within the acid-digestion methodologies employed. In this study, acid digestion was performed in a closed vessel on a traditional hot plate; however, most other studies in Table 3 used a closed-vessel microwave system. Acid digestion in a microwave allows for greater digestion as the samples are digested at much higher temperatures and pressures. Despite these methodological variations, this study found no difference between HNO_3 and H_2O_2, and HF and HNO_3 digestions on krill Fe concentrations (Figure 4.1), suggesting that methodological differences may not be driving the large variability observed in the literature.

Lastly, the different analytical instruments as well as the trace metal clean protocols used, could contribute to lower or higher Fe levels. Although most studies reported in Table 4.3 used an ICP-MS, use of a Sector Field ICP-MS (e.g. Barbante et al. 2000, Nicol et al. 2010, Palmer et al. 2006, and this study) allows for interference free Fe measurements at higher resolution compared

to standard quadropole ICP-MS units (e.g. Kim et al. 2014 used an Elan 6100 ICP-MS). Other instruments used included ICP – Atomic Emission Spectroscopy (e.g. Caroli et al. 1998 and Deheyn et al. 2005) and Flame Atomic Absorption Spectroscopy (Locarnini and Presley 1995). Analytical instruments are not expected to be major contributors to large variations observed in the literature.

4.5.4 Iron-to-carbon ratio

The higher Fe/C ratio in the stomach and digestive gland compared to the rest of the body suggests that krill are ingesting more Fe than they require. The Fe/C ratio for whole krill from this study (10.3 ± 4.3 μmol mol^{-1}) is much lower compared to our previous estimate (69.0 μmol mol^{-1} in Ratnarajah et al. 2014) based on samples collected at a different location (Table 4.3). Iron-to-carbon ratios in whole krill from the recent study are also similar to diatoms (6 μmol mol^{-1}), autotrophic flagellates (8.7 μmol mol^{-1}) and heterotrophic flagellates (14.1 μmol mol^{-1}) growing in Fe deplete conditions (Twining and Baines 2004).

4.5.5 Antarctic krill as a biological reservoir of iron in the Southern Ocean

Based on the relatively low measured Fe concentrations and Fe/C ratios in whole krill compared to other published studies, we investigated a number of potential drivers of the large variation reported in the literature. Four potential sources of this variation were examined, namely varying ingestion and moulting rates (both considered as physiological factors), along with seasonal and regional effects, (considered as external factors).

4.5.6 Physiological factors influencing iron concentration

As the dominant herbivore in the Southern Ocean, Antarctic krill can consume up to 15% of its body carbon weight day^{-1} (mean body wet weight = 0.486 g) (Pakhomov et al. 2002). Ingestion rates have been reported as being higher over spring and summer when phytoplankton is abundant (Schmidt et al. 2012). Our results suggest that there was a high intake of Fe-rich food at the time of collection for all three years as evidenced by the high Fe concentrations measured

in the stomach (Table 4.2). The large range in Fe concentrations in krill stomach is most likely due to varying individual ingestion rates.

In the stomach, the food is ground and mixed with digestive enzymes from the digestive gland (Saborowski and Buchholz 1999). The ground food then passes through a primary filter; a comb-like filter that retains coarse food particles (Ulrich et al. 1991). Only colloids (fine food particles and soluble materials ~ <0.1 μm) pass through the primary filter into the posterior part of the stomach (Ulrich et al. 1991, Saborowski and Buchholz 1999). Residual insoluble material is passed down the hindgut and defecated as faecal pellets. Consequently, the high daily ingestion rates of krill could lead to more rapid sinking of phytoplankton compared to natural senescence through uneaten phytoplankton, and passive sinking. The soluble material passes through a secondary filter into the digestive gland where nutrient absorption takes place (Ulrich et al. 1991, Saborowski and Buchholz 1999).

In this study, mean Fe concentrations in the digestive gland are only slightly lower than those found in the stomach (except for Feb 2003), but are still much higher than those of the abdominal muscle (Table 4.2). This indicates that some Fe is being absorbed and utilised by the krill, but the muscles require little Fe. Therefore, much of the Fe in krill is not locked up in the muscle, but instead processed and ingested daily. Mean Fe concentrations in the exoskeleton and krill body (excluding stomach and digestive glands) were higher than those observed for the abdominal muscle, suggesting that Fe may also be required for the growth and production of the exoskeleton (and other parts of the krill not individually measured here, e.g. head, thoracopods (filtering appendages), pleopods (swimming appendages) and telson (tail) (Table 4.2).

Moulting in Antarctic krill occurs between 13 and 30 days over summer, and up to 90 days over winter, and is dependent on temperature and food availability (Poleck and Denys 1982, Ikeda and Thomas 1987, Buchholz 1991, Nicol and Stolp 1991, Kawaguchi et al. 2006). The higher Fe concentration in the exoskeleton compared to the muscle in this study suggests that Fe may be required for the production of new exoskeleton. However, the source of Fe present in the

exoskeleton could either be: 1) absorbed from the diet for physiological requirements, 2) absorbed directly from the seawater for physiological requirements, or 3) adsorbed from the seawater onto the exoskeleton and not required for physiological processes. Absorption of fluoride directly from the seawater into the exoskeleton of krill has been demonstrated previously (Nicol and Stolp 1991) to satisfy the high physiological requirement of fluoride (up to 2.6 μg mg^{-1} in the exoskeleton reported by Adelung et al., 1987).

However, the higher Fe/C ratio in the digestive organs compared to the muscle found in this study, as well as the high Fe concentration in krill faecal material, suggests that krill are taking up more Fe than they require, and consequently they would not need to absorb Fe directly from the seawater. Because the concentration of Fe in the exoskeleton is low, and faecal Fe concentrations are high, it is proposed that the Fe present in the exoskeleton is a combination of absorption through the diet and indirect adsorption from the seawater. In addition, because there is a significant amount of iron in the exoskeleton, regular moulting by krill could contribute to the stripping of Fe from seawater down to seabed due to the sinking of discarded moults.

4.5.7 External factors influencing iron concentration

4.5.8 Seasonal effects

Krill obtain Fe through their diet and their diet changes seasonally. Krill are primarily herbivores in spring and summer (Pakhomov et al. 2002), but may also be omnivorous (Schmidt et al. 2014). Krill have a more omnivorous diet over autumn and winter (Kawaguchi et al. 1986, Spiridonov 1992), and may have a reduced feeding rate and a concomitant decrease in respiration rates (Quentin and Ross 1991, Torres et al. 1994, Atkinson et al. 2002, Meyer et al. 2010) or tolerate starvation and shrink if deprived of food for long periods (Ikeda and Dixon 1982).

The monthly concentration of Fe in krill and is highest in March (190.5 ± 0.4 mg kg^{-1}), lower in April (66.7 ± 0.3 mg kg^{-1}) and lowest in August (36.9 mg kg^{-1}) (Table 4.3, Kim et al. 2014). This decline in Fe concentration in whole krill is consistent with a diet that is rich in Fe during the summer months when they are feeding on algae but a reduced Fe diet in winter when they may be utilising other food sources, combined with starvation.

4.5.9 Regional effects

There are some regional differences in Fe concentration in krill. Antarctic krill along 13 stations in the Western Antarctic Peninsula exhibited variations in Fe concentration in whole krill between 4 – 81 mg kg^{-1} (Locarnini and Presley 1995). These variations may reflect regional difference in diet.

In our study, the ingested Fe in the digestive organs is almost entirely of soluble origin as the digestions with HF added showed no difference compared to those where HF was absent (Figure 4.1). However, krill from the Scotia Sea demonstrated a high refractory load (Schmidt et al. 2011), with stomach Fe concentration ~ 1000 times greater than our krill stomachs. The difference could be a result of ingestion of refractory particles through benthic feeding in the Scotia Sea (Clarke and Tyler 2008). Despite the high Fe concentration of the krill stomachs containing refractory Fe, the mean muscle Fe concentration was similar to measured values here (3.2 mg kg^{-1} in Schmidt et al. 2011 compared to 5 ± 1 mg kg^{-1} in our study). This suggests that fully-grown krill require little Fe, and are ingesting excess Fe with no metabolic need via feeding habits.

If the majority of Fe ingested by krill has no metabolic function, then could the high Fe concentration in krill stomach observed by Schmidt et al. (2011) influence the Fe concentration in whole krill to the extent that it could account for the range of values reported in the literature? We examined the potential effect of the Fe concentrations in the digestive organs (stomach and digestive gland) on the total Fe concentration of whole krill. The stomach represents 1.2% (±

0.4%) of the total dry weight of an individual krill, the digestive gland represents 6.1% (± 2.4%) and the rest of the krill body represents 92.7% (± 2.6%) (n = 10).

Using mean Fe concentration values for stomach, digestive gland and the rest of the krill body from this study (Table 4.2), and based on the relative weight proportion measured above, the total Fe concentration in whole krill was calculated to be 12 mg kg^{-1} (February 2003), 10 mg kg^{-1} (March 2012), and 12 mg kg^{-1} (February 2015). This is similar to the mean values for whole intact krill without dissection (19 ± 7 mg kg^{-1} from February 2003, 10 ± 3 mg kg^{-1} from March 2012, and 18 ± 12 mg kg^{-1} from February 2015, Table 4.2). The difference in values between the calculated and intact whole krill could be due to loss of the body fluids during dissection.

If the Fe concentration in krill stomach and muscle from Schmidt et al. (2011) are substituted into the calculations Fe concentration above, using their stomach concentrations (2783 mg kg^{-1}) for the digestive gland (they did not report iron values for this organ), then whole krill would have Fe concentrations of 206 mg kg^{-1}. This could be an overestimate because Schmidt et al. (2011) did not provide measurements of the iron concentration of digestive gland. Even if the Fe concentration in the digestive gland were half what was in the stomach from their samples, the total Fe concentration of whole krill would be 121 mg kg^{-1}.

The Fe measurements in krill (Table 4.2, Figure 4.2) and calculations above clearly indicate that 1) krill are ingesting more Fe in their diet than they require, 2) feeding location is an important factor influencing Fe concentrations in krill stomachs (e.g. krill feeding in Fe-richer areas such as the seabed would demonstrate higher Fe concentration in the stomach than surface feeding krill) and 3) high Fe concentration in the stomach and digestive gland could significantly contribute to the determined Fe concentration found in whole krill, with the digestive gland having greater influence because of its relatively large size compared to the stomach.

4.6 Conclusions

4.6.1 Understanding the variation in iron concentration in krill

Krill act as a reservoir of Fe in Southern Ocean surface waters, but the size of this reservoir is dependent on a range of factors. The results from this study demonstrate that a large portion of total Fe is found in the digestive organs (stomach and digestive gland). Generally, krill ingest more Fe than is required for physiological processes. This is evident in the high Fe concentration in the stomach compared to the digestive gland or rest of the body. Varying ingestion and assimilation rates can lead to natural variability in the Fe concentrations within body parts and whole individuals. The variability in Fe concentrations observed in whole krill could also be due to feeding in Fe-rich areas, reflected in higher Fe concentrations of the digestive organs, which affects whole body levels. The range in iron values in the literature is unlikely to be the result of analytical or methodological differences.

4.6.2 Implications for the transfer of iron to higher trophic levels

Biological recycling of Fe by higher order marine animals in the Southern Ocean is a relatively new field but is receiving considerable attention (Smetacek and Nicol 2005, Tovar-Sanchez et al. 2007, 2009, Nicol et al. 2010, Schmidt et al. 2011, Smith et al. 2013, Ratnarajah et al. 2014, Wing et al. 2014, Ratnarajah et al. 2016a). The wide range of Fe concentrations in whole krill (4 – 190 mg kg^{-1}) is the most influential parameter in determining the recycling efficiency of blue, fin and humpback whales on primary productivity in the Southern Ocean (Ratnarajah et al. 2016a).

Iron concentrations in krill in our study were relatively low, with a narrow range but they were obtained from a relatively restricted area and from a similar time of year. Other published studies have demonstrated consistently high Fe values, and decreasing whole body Fe concentrations as winter approaches. Thus a better understanding of spatial and temporal variation in Fe concentration of krill needs to be incorporated into any diet-dependent model that investigates the effect of higher order marine animals on primary productivity. Samples of krill (trace metal

clean) need to be collected from as wide a range of areas as possible and from throughout the year to clarify the temporal and spatial variations in the iron concentration of krill.

4.7 Acknowledgements

We would like to thank Fanny Chever, Pier van der Merwe, Rob King, Matthieu Bressac, Jess Melbourne-Thomas and Tom Trull for their comments and suggestions during the development of this project and manuscript. Thomas Rodemann of the Central Science Laboratory is acknowledged for performing the carbon determinations. Lastly, to Tino Bowie-Proemse for insightful discussions and a good game of catch. This study was supported by the Australian Government's Cooperative Research Centres Programme through the Antarctic Climate and Ecosystem Cooperative Research Centre, the Institute for Marine and Antarctic Studies, the Holsworth Wildlife Research Endowment and the European Union COST Action ES0801. Access to ICP-MS instrumentation was supported through ARC LIEF funds (LE0989539).

5 Physical speciation and solubility of iron from baleen whale faecal material

This chapter has been submitted:

Ratnarajah L, Lannuzel D, Townsend AT, Meiners KM, Nicol S, Friedlaender AS, Bowie AR. (in revision) Physical speciation and solubility of iron from baleen whale faecal material. Marine Chemistry

5.1 Abstract

Primary productivity in large areas of the Southern Ocean is limited by the availability of a key micronutrient – iron (Fe). Recently it has been suggested that marine animals could play an important role in recycling Fe through their diet and subsequent defecation, however there is no information on the relative bioavailability of faecal Fe for uptake. The bioavailability of Fe in seawater is controlled by a number of complex interactions. The physical separation between the dissolved (<0.2 μm) and particulate (>0.2 μm) fractions is one common measure used to determine element bioavailability. Here, the size fractionation of Fe from 3 whale faecal samples in 4 different size classes (<0.2 μm, 0.2 – 10 μm, 10 – 60 μm and >60 μm) was investigated, along with the leaching of particulate Fe over time. Although the total particulate fraction (>0.2 μm, 5,026 – 22,526 nmol L^{-1}) dominated the total Fe pool, the concentrations of dissolved Fe in whale faecal samples (186 – 754 nmol L^{-1}) were three order of magnitude higher than published Southern Ocean surface seawater concentrations. Furthermore, results from the leaching experiment suggest that Fe is continually leached from faecal particles over an initial 12-hour period, thus increasing the concentration of bioavailable Fe in surface seawater. Although the concentrations measured here are some of the highest reported in the literature, the true supply of Fe back to surface seawater will be controlled by processes such as organic complexation, scavenging and sinking by particles, remineralisation, and vertical transport, not measured in this study.

5.2 Introduction

Large regions of the Southern Ocean are characterized by low phytoplankton biomass despite high concentrations of major nutrients (e.g. nitrate, phosphate and silicate), and have been characterised as High Nutrient Low Chlorophyll (HNLC) waters (Moore and Abbott 2000). One factor responsible for limiting the growth of phytoplankton in HNLC waters has been the availability of iron (Fe) that is required for nitrogen acquisition and assimilation (Morel and Price 2003). *In-situ* Fe fertilisation experiments and bottle assays have demonstrated that Fe deficiency limits the ability of phytoplankton to acquire nitrogen, consequently limiting photosynthetic yield (Martin 1990, de Baar et al. 2005, Boyd et al. 2007).

Iron enters the surface waters through the deposition of atmospheric dust (Boyd et al. 2004, Cassar et al. 2007), weathering of shelf sediments (Sedwick et al. 2008, Bowie et al. 2009), hydrothermal vents (Tagliabue et al. 2010, Klunder et al. 2011), melting icebergs (Smith et al. 2007, Lin et al. 2011, Duprat et al. 2016) and sea ice (Sedwick and Di Tullio 1997, Lannuzel et al. 2007), and upwelling of nutrient rich deep water (de Baar et al. 1995). However, the concentration of Fe in Southern Ocean surface waters is at limiting concentrations (0.1 – 0.5 nmol L^{-1}, Tagliabue et al. 2012) due to the remoteness of large areas of the Southern Ocean to many of these sources, and the short residence time of Fe in surface waters (Millero et al. 1987). Recently, it has been proposed that biological recycling by higher order marine animals, in particular baleen whales, could increase the availability of Fe to phytoplankton (Smetacek and Nicol 2005, Nicol et al. 2010, Smith et al. 2013, Ratnarajah et al. 2014, Ratnarajah et al. 2016a, Ratnarajah et al. 2016b).

Antarctic krill *(Euphausia superba)* concentrate Fe through their diet (phytoplankton, copepods etc.) which is influenced by seasonal and regional differences, as well as surface or benthic feeding (Ratnarajah et al 2016b). Baleen whales feed on Fe-rich Antarctic krill *(Euphausia superba)* as their main dietary source (Lockyer 1981, Nicol et al. 2010). Whales require Fe for the production of red blood cells and the oxygen storage protein in muscles (Ordway and Garry 2004, Ganz and Nemeth 2006); however, mammals are unable to excrete Fe through their

kidneys. The high concentrations of total Fe in whale faeces (119 ± 30 mg kg^{-1} dry weight for humpback whales, 162 ± 107 mg kg^{-1} dry weight for blue whales and for 237 ± 45 mg kg^{-1} dry weight fin whales, Nicol et al. 2010, Ratnarajah et al. 2014) demonstrate that it is a source of recycled Fe. However, not all Fe in the ocean is bioavailable and it is unclear if this recycled Fe is bioavailable for uptake by phytoplankton.

The bioavailability of Fe reflects the ability of phytoplankton cells to acquire Fe; with the dissolved Fe (dFe, operational cut-off of 0.2 or 0.4 μm) fraction being considered most accessible for biological uptake despite the particulate Fe (pFe) fraction being the dominant pool of total Fe in the water column (de Baar and de Jong 2001). However, the particulate fraction could also be an important reservoir that can be solubilized (Wu et al. 2007, Schroth et al. 2009, Sugie et al. 2013). Multiple leaching experiments have been conducted to explore the dissolution of Fe under widely varying conditions (e.g. ultrapure deionized water leaching, weak acid, seawater leaching and semi-continuous flow-through leaching techniques) (Wu et al. 2007, Schroth et al. 2009, Aguilar-Islas et al. 2010, Mendez et al. 2010, Gao et al. 2013, Winton et al. 2015). These studies demonstrated the need for including the dissolved and particulate fractions, and the leachable fraction, in determining the total amount of Fe in seawater that is potentially bioavailable for uptake by phytoplankton.

Biological recycling of Fe by baleen whales could represent a large source of Fe into surface waters, however there is no information on the relative potential bioavailability of faecal Fe in surface waters. The overall aim of this study is to provide the first quantitative estimate of the fraction of dFe to pFe in whale faeces, and the solubility of this recycled Fe source over time. Dissolved Fe respresents the fraction passed through a 0.2 μm pore size filter as a means to partition dFe and pFe. The solubility represents the concentration of dissolvable Fe from pFe at each size fraction. The dissolvable Fe may contain labile Fe^{2+}, organically complexed Fe^{3+}, and colloidal (between 0.02 μm to 0.2 or 0.4 μm) Fe (Siefert et al. 1999, Trapp et al. 2010). Aluminium is commonly used as a tracer of lithogenic inputs (Ohnemus and Lam 2014). Therefore the concentration of aluminium (Al) was also measured to determine the Fe/Al ratio to

estimate whether the pFe in whale faeces is more likely to originate from biogenic or lithogenic sources.

5.3 Methods

5.3.1 Sample preparation

New 20 L low-density polyethylene (LDPE) carboys, 1 L LDPE bottles, silicon C-flex tubing, and 5 and 10 mL LDPE nutrient tubes were soaked in 2% Decon 90 (Decon Laboratories) cleaning solution for at least 7 days. Subsequently carboys, bottles, tubing, and nutrient tubes were acid leached in 10% (v:v) Hydrochloric acid (HCl, Merck, Analytical grade 32%, Germany) for 4 weeks prior to use for transferring freshly collected sub-Antarctic seawater samples for leaching experiment and the dilution and analysis of size fractionated samples. Teflon perfluoroalkoxy (PFA) screw cap digestion vials (15 mL; Savillex Corp., USA) were acid leached in 50% (v:v) HCl for 2 weeks. Following acid leaches, all materials were rinsed thoroughly (five times) with Ultra High Purity (UHP) water and left to dry in an HEPA filtered Class 100 laminar flow bench.

60 μm (Nylon net filters, 47 mm, Merck Millipore), 10 μm and 0.2 μm membrane filters (polycarbonate (PC), 47 mm diameter, Sterlich and Merck Millipore, respectively) were soaked in 10% ultrapure HCl for 1 week, rinsed with UHP water 7 times, and stored in UHP water. Polycarbonate filtration units (Sartorius) were soaked in 2% Neutracon (Decon Laboratories) cleaning solution for 7 days and acid leached in 10% (v:v) HCl (Merck, Analytical grade 32%, Germany) for 3 days prior to use. Following acid leaches, all filtration units were rinsed thoroughly (five times) with UHP water and left to dry in a HEPA filtered Class 100 laminar flow bench.

5.3.2 Sample collection

Adult humpback whale faecal samples from 3 individuals, were collected opportunistically using new Nalgene 250 mL LDPE bottles in Antarctic waters during the 2014/2015 summer feeding season around Palmer Station, Anvers Island. Whale faeces are mostly liquid in nature, with some solid particles (Figure 1b). Faecal samples were collected by dipping a Nalgene LDPE

bottle into seawater from a 6-meter inflatable boat, as the whale was simultaneously defecating and then diving, and immediately frozen. Each sample consists of a mixture of faecal material and seawater, as it is not possible to solely collect the liquid fraction of whale faeces.

Seawater used for leaching experiments were collected from a HNLC region using a SeaBird trace metal clean rosette equipped with 12 x 10 L externally closing Ocean Test Equipment Teflon-lined Niskin bottles, attached to a polyurethane powder-coated aluminium frame specially designed for trace metal work during the Heard Earth-Ocean-Biosphere Interactions (HEOBI) cruise (8 January – 5 March 2016) in the vicinity of Heard Island, Southern Ocean. Bottles were tripped at pre-programmed depths using a pressure sensor. Seawater used for the leaching experiments was collected at a depth of between 28 – 83 m. Seawater samples were drawn through C-flex tubing and filtered in-line through 0.2 μm pore-size acid-washed capsules (Pall Supor membrane Acropak 200). The dissolved fraction of the seawater collected is thus a combination of the colloidal and soluble (<0.02 μm) fraction. All sample processing was carried out under an ISO class 5 trace-metal-clean laminar flow bench in a HEPA filtered-air clean container.

5.3.3 Analysis of iron and aluminium

10 mL of faecal material from each humpback whale was sequentially vacuum filtered through 47 mm diameter acid washed 60 μm, 10 μm, and 0.2 μm membranes to obtain the dissolved and particulate fractions. This volume of faecal material was chosen because high particle concentrations restrict the volume of sample that is able to be passed through the filters before clogging.

The dissolved and particulate fractions were measured using Sector Field Inductively Coupled Plasma Mass Spectrophotometry (SF-ICP-MS, Thermo Fisher ELEMENT 2 Bremen Germany), following methods described in Townsend (2000) and Bowie et al. (2010). Briefly, particulate samples were measured by digesting filters in 1 mL of concentrated nitric acid (HNO_3), 1 mL of concentrated hydrofluoric acid (HF) and 0.125 mL of hydrogen peroxide (H_2O_2) (all Ultrapure,

Seastar Baseline, Choice Analytical) inside Teflon PFA vials. Faecal material contains high concentrations of dissolved organic material. The addition of H_2O_2 enhances the oxidation properties of HNO_3 (especially in the digestion of organics), whilst the addition of HF allows for greater digestion of the Fe particles retained on the filters compared to HNO_3 and H_2O_2 assisted digestion only (Sucharová and Suchara 2006, Bowie et al. 2010, Ohnemus et al. 2014). Digests were heated at 125°C for 8 hours on a Teflon coated digestion hotplate, housed in a bench-top fume hood coupled with HEPA filters to ensure clean air input (Digiprep, France).

Following digestion, all samples were dry evaporated at 60°C for 4 hours on the Teflon coated digestion hotplate, cooled for ~2 hours, then resuspended in 10 mL of 10% v:v HNO_3 (Ultrapure, Seastar Baseline) for 2 days. Identical procedures were applied to procedural filter blanks (n = 9) and to one certified referenced material (BCR-414 trace elements in plankton, n = 3) to assess elemental recovery following digestion. Prior to analysis all particulate samples, certified reference materials (CRMs) and digest blanks were diluted 10-fold in 2% v:v HNO_3 (Ultrapure, Seastar Baseline) in LDPE nutrient tubes. The dissolved fraction was diluted 10-fold in 2% v:v HNO_3 (Ultrapure, Seastar Baseline) in LDPE nutrient tubes to reach final salinity <3, thereby minimising sample matrix effects during SF-ICP-MS analysis.

Indium (In, High-Purity Standards, USA) was added to all samples at a final concentration of 10 μg L^{-1} and used as an internal standard. Representative subsamples from each analytical sequence were also spiked with a multi-element solution (QCD Analysts, MISA suite of solutions, 10 μg L^{-1}, Spring Lake, USA) to monitor the recoveries of Fe and Al in the sample matrices considered. The ELEMENT 2 SF-ICP-MS has three pre-defined spectral resolutions available enabling isotopes to be quantified with minimal spectral interferences. Iron and Al were measured using the “medium” resolution setting ($m/\Delta m \sim 4000$). Four calibration standards with concentrations 0, 1, 5 and 10 μg L^{-1} were prepared by serial dilution from multi-element stock solutions (QCD Analysts, MISA suite of solutions, Spring Lake, USA).

5.3.4 Leaching of particulate iron

Background dFe concentration of the HEOBI seawater used for this experiment was determined using a sample pre-concentration and matrix separation instrument for undiluted seawater (seaFAST S2, Elemental Scientific, USA) and subsequently measured on the SF-ICP-MS. Dissolved Fe concentration was determined to be 0.17 ± 0.02 nmol kg^{-1} (n = 7). Laboratory analysis of SAFe intercalibration sample D1 for dFe was determined to be 0.69 ± 0.05 nmol kg^{-1} (n = 7). This agrees with the consensus value for this standard of 0.67 ± 0.04 nmol kg^{-1} (Wuttig et al. in prep).

Sample 3 was randomly chosen for the leaching experiment. Initially, a fresh 10 mL subsample of faecal material was sequentially filtered through acid washed 60 μm, 10 μm, and 0.2 μm filters using the filtration devices coupled with a vacuum pump (Merck Millipore). 50 mL of filtered (0.2 μm) seawater was then added into the filtration chamber. Filters were soaked in this solution for 5 minutes, and 1 mL of the seawater and faecal material solution was subsequently filtered using a vacuum pump. Following that, 1 mL of the seawater and faecal material solution were filtered at 15, 30, 60 minute, and 4 and 12 hour intervals. The experiment was conducted in the dark at ambient seawater temperature (2°C) to mimic natural conditions. At the end of the experiment, the filters were digested and analysed as above. The seawater and faecal Fe leacheates were diluted 20-fold in 2% HNO_3 in LDPE nutrient tubes with 10 μg L^{-1} In added as internal standard (as above).

5.4 Results

5.4.1 Analysis of iron and aluminium

Results for procedural blanks, limits of detection (defined as three times the standard deviation of the blank) and certified reference materials are presented in Table 5.1 and 5.2 respectively, and were found fit for purpose. Spike recoveries of Fe in faecal digests were between 83 – 96% (n = 7).

Whale faecal material is voluminous (Figure 5.1a) and highly heterogeneous in nature, consisting of a mixture of dissolved, small and large particles (Figure 5.1b). Measured dFe and pFe concentrations for the 3 samples are presented in Table 5.3 (in nmol L^{-1}) and Figure 5.2 (in %). The concentration of dFe in whale faecal material ranged from 186 – 754 nmol L^{-1} (Table 5.3 and Figure 5.2), which is comparable to marine and continental ice in East Antarctica (Table 5.4). The particulate fractions were individually measured at 3 different size fractions (0.2 –10, 10 – 60 and >60 μm) (Table 5.3 and Figure 5.2). The concentration of size-fractionated pFe varied greatly between each faecal sample and between each size fraction, ranging from 380 – 2,508 nmol L^{-1} for the 0.2 – 10 μm fraction, 703 – 1,353 nmol L^{-1} for the 10 – 60 μm fraction, and 1,815 – 20,784 nmol L^{-1} for the largest particles (>60 μm).

The >0.2 μm fraction is the most commonly reported particulate fraction in the literature. 'Total pFe' in Table 3 was determined by summing the Fe concentrations measured in the 0.2 – 10, 10 – 60 and >60 μm fractions. Total particulate fraction in whale faecal samples ranged from 5,026 – 22,526 nmol L^{-1} (Table 5.3), which is comparable to marine ice, fast ice, hydrothermal vents and krill faecal pellets (Table 5.4). 'Total Fe' represents the sum of the dissolved (< 0.2 μm) and particulate (> 0.2 μm) fractions, which ranged from 5,780 – 23,053 nmol L^{-1} (Table 5.3). The particulate fraction consistently dominated the total Fe pool (87 – 99 %, Table 5.3). Assuming that Al is solely lithogenic in origin, the pFe/pAl in whale faecal samples suggest that the pFe in whale faecal samples is highly biogenic in origin (between 50 – 80%).

5.4.2 Leaching of particulate iron

Although dFe is generally considered the most bioavailable fraction, Fe can be leached from the particulate fraction into the dissolved fraction over time. This fraction of leached Fe is overlooked in instantaneous size fractionation measurements.

At the start of the 12-hour leaching experiment, the total Fe concentrations on the 0.2 μm, 10 μm and 60 μm filters were 1,021 nmol L^{-1}, 1,557 nmol L^{-1} and 17,229 nmol L^{-1}, respectively. Initial dFe of the whale faecal material was measured at 207 nmol L^{-1}.The pFe concentrations presented

here were determined by summing the final Fe concentration in the filters, and at each subsampling time step as measured on the SF-ICP-MS.

The concentrations here are different from that presented for Sample 3 in Table 5.3 due to the inherent heterogeneity of the sample and the heavy particle load that would influence individual sub-sampling attempts (Figure 5.1b). The leaching experiment demonstrated that most of the labile Fe within each size fraction is released within the first 5 minutes (1 – 7 %, Figure 5.3). After 12 hours, between 0 – 2.5% of Fe was still being leached by the faecal particles.

Table 5.1 Measurements of Fe and Al from procedural filter blanks. Averages shown are the mean of 3 replicates. The limit of detection (LOD) is 3 times the standard deviation of the filter blank.

	Fe	**Al**
0.2 μm		
Average filter blank (μg L^{-1})	0.7 ± 0.1	1.1 ± 0.2
LOD (μg L^{-1})	0.2	0.5
10 μm		
Average filter blank (μg L^{-1})	1.1 ± 0.09	0.4 ± 0.06
LOD (μg L^{-1})	0.3	0.2
60 μm		
Average filter blank (μg L^{-1})	0.7 ± 0.3	1.1 ± 0.2
LOD (μg L^{-1})	1.0	0.5

Table 5.2 Analysis of certified referenced material plankton (BCR-414) for Fe and Al. Reference values for Fe are certified, but Al values are indicative only. Averages shown are the mean of 3 replicates. Recovery values indicate the percentage difference between measured and certified values.

	Fe	**Al**
BCR-414 referenced values (mg kg^{-1})	1,850 ± 190	[1,800 ± 30]
Measured average (mg kg^{-1}) (n= 3)	1,709 ± 22	2,563 ± 46*
Recovery (%)	92	142

*[Al] is a non-certified value only. Similarly high values for Al (2639 ± 80) were also found using a combination of HCl, HF and HNO_3 under similar digestion conditions by Lannuzel et al. (2014).

Table 5.3 Iron concentration in nmol L^{-1} in the different size fractions, and percent (%) pFe (>0.2 μm) for each sample of whale faecal material. Total pFe represents the sum of iron concentrations >0.2 μm, whilst Total Fe represents the sum of all size fractions.

	Sample 1	**Sample 2**	**Sample 3**
<0.2 μm	754	527	186
0.2 μm	2,508	389	486
10 μm	703	1,353	1,212
60 μm	1,815	20,784	17,664
Total pFe	*5,026*	*22,526*	*19,362*
% pFe	*87*	*98*	*99*
Total Fe	*5,780*	*23,053*	*19,548*

Table 5.4 Summary of dissolved iron (dFe) and particulate iron (pFe) concentrations from various sources in the Southern Ocean.

Location	Source	Fraction	Fe (nmol L^{-1})	Reference
Southern Ocean	Humpback whale faeces	dFe[a]	186 – 724	*This study*
	Surface seawater	dFe[a]	0.1 – 0.5	Tagliabue et al. 2012
Polar Frontal region	Surface seawater	dFe[a]	0.5 – 3.5	de Baar et al. 1995, Löscher et al. 1997
	Deep water	dFe[a]	0.4 – 2.8	Löscher et al. 1997
East Antarctica	Pack ice	dFe[a]	0.2 – 26	Lannuzel et al. 2007, van der Merwe et al. 2009, van der Merwe et al. 2011a,
	Fast ice	dFe[a]	0.9 – 7.1	Van der Merwe 2011b
	Snow	dFe[a]	1 – 31.7	Edwards and Sedwick 2001, Lannuzel et al. 2007
	Brine	dFe[a]	4.7 – 25.5	Lannuzel et al. 2007
	Marine ice	dFe	339 – 691	Herraiz-Borreguero et al 2016
	Continental ice	dFe	62 - 167	Herraiz-Borreguero et al 2016
Ross Sea	Sea ice	dFe[a]	1.1 – 6	Grotti et al. 2005
Taylor Glacier and Canada Glacier, Weddell Sea	Glacier	dFe[a]	0.1 – 2.2	Raiswell et al 2008
Seymour Island and King George Island, Weddell Sea	Iceberg	dFe[a]	0.05 – 3.8	Raiswell et al 2008
Antarctic Circumpolar Current (56°S, 15°W)	Deep water upwelling	dFe[b]	1	de Baar et al. 1995
Taylor Valley	Antarctic stream[d]	dFe[c]	190	Lyons et al. 2015
Bransfield Strait	Hydrothermal	dFe[b]	2.2 – 12.6	Klinkhammer et al. 2001
Southern Ocean	Humpback whale faeces	pFe	5,026 – 22,526	*This study*
East Antarctica	Pack ice	pFe	0.1 – 213	Lannuzel et al. 2007, van der Merwe et al. 2009, van der Merwe et al. 2011a
	Fast ice	pFe	40.39 – 10,385	Van der Merwe et al. 2011b, Lannuzel et al. 2014
	Surface snow	pFe	0.1 – 18.3	Lannuzel et al. 2007
	Brine	pFe	0.5 – 12	Lannuzel et al. 2007
	Marine ice	pFe	13,323 – 14,679	Herraiz-Borreguero et al 2016
	Continental ice	pFe	26 – 31	Herraiz-Borreguero et al 2016
Ross Sea	Sea ice	pFe	26 – 1,160	Grotti et al. 2005
	Aeolian deposition	pFe	0.7 - 430	Winton et al. 2015 and Bowie et al. 2009
East Scotia Ridge	Hydrothermal	pFe 0.2	820,000 – 1,312,000	James et al. 2014
Pyrdz Bay	Krill faecal pellet	pFe	12,234,757 – 18,612,163[e]	Ratnarajah et al. 2016b

[a] dFe is <0.2 μm

[b] Unclear if dFe is <0.2 μm or <0.4 μm

[c] dFe is <0.4 μm

[d] Potential sources of iron into the stream include chemical weathering of the stream channel sediments or aeolian inputs on glacial surfaces

[e] pFe concentration in nmol kg^{-1} dry weight

Table 5.5 Iron-to-Aluminium molar composition and estimated lithogenic and biogenic contributions in the three faecal samples (for details see text). A comparative crustal Fe/Al ratio of 0.2[a] was used was used for calculations.

	[pAl] nmol L^{-1}	**[pFe] nmol L^{-1}**	**Fe/Al**	**Lithogenic [pFe] nmol L^{-1}**	**Biogenic [pFe] nmol L^{-1}**	**Lithogenic (%)**	**Biogenic (%)**
Sample 1	12,674	5,026	0.40	2,535	2,491	50	50
Sample 2	22,677	22,526	0.99	4,535	17,991	20	80
Sample 3	24,454	19,362	0.79	4,891	14,471	25	75

[a] Wedepohl (1995)

[b] pFe and pAl is >0.2 μm

a)

b)

Figure 5.1 a) Defecating humpback whale in the Southern Ocean (source A.S Friedlaender), b) Close-up of 5 mL of humpback whale faecal material from sample 3.

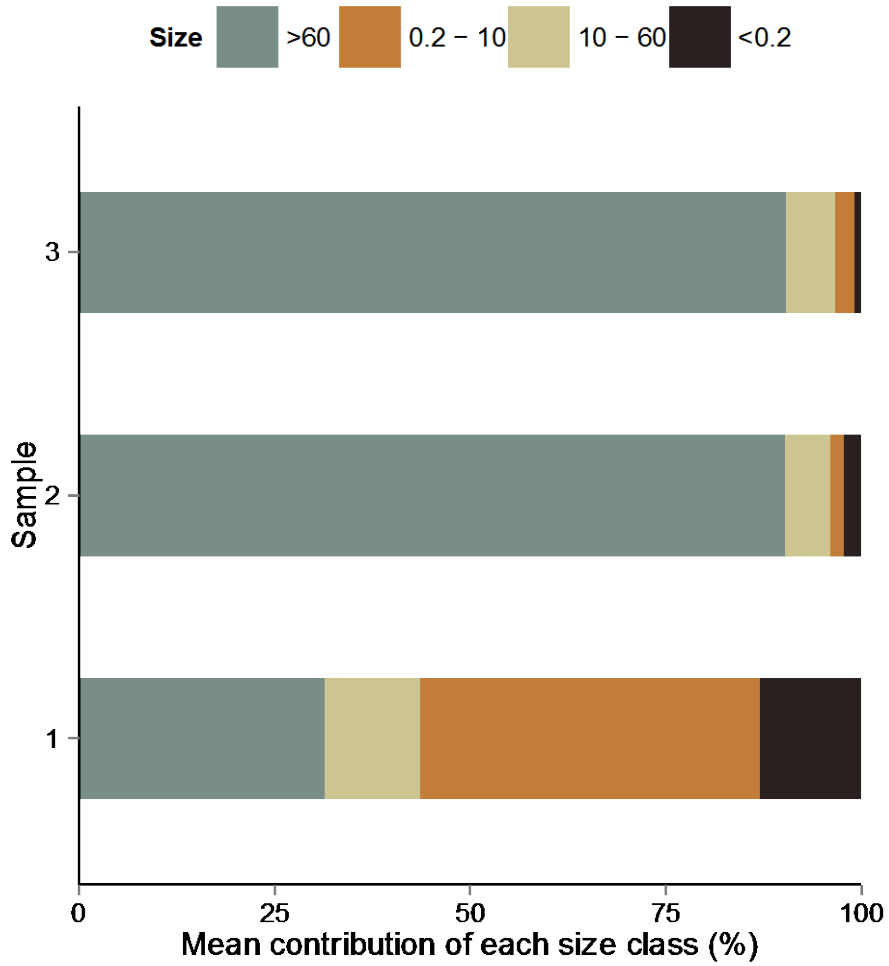


Figure 5.2 Mean percent (%) contribution of each size fraction (µm) to total iron in whale faecal material.

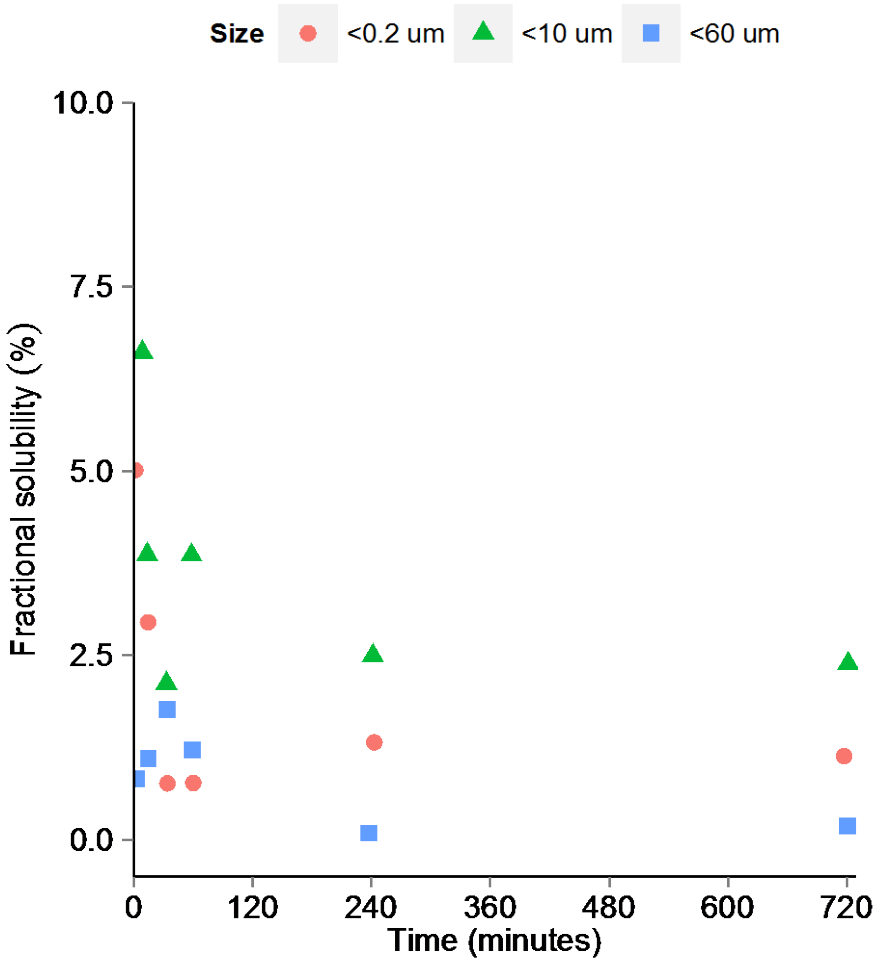


Figure 5.3 Leached Fe from whale faecal material as a function of time in each size fraction (Sample 3).

5.5 Discussion

5.5.1 Dissolved fraction (<0.2 μm)

The highly heterogeneous nature of whale faecal material would influence the apparent variability of the measured dFe concentrations between these samples (186 – 754 nmol L^{-1}) because some faecal material might have more particulate matter in the volume sampled (Figure 5.1b). However, the dFe concentrations in whale faecal material fall within the range of other dFe pools found in the Southern Ocean region (Table 5.4). The dFe concentrations measured in our samples are most comparable to marine ice and continental ice from the Amery Ice Shelf, which demonstrate similarly wide ranges in Fe concentrations (339 – 691 nmol L^{-1} and 62 – 167 nmol L^{-1} respectively), but much higher than in other pools, including background seawater concentrations from the Southern Ocean and the Polar Frontal region, sea ice, snow, glaciers, icebergs, Antarctic streams, upwelled subsurface waters, and hydrothermal sources (Table 4).

The large variability in dFe concentration measured here could also reflect the timing of sample collection (i.e. between defecation and collection) and the variability in ingested food quantity and quality. Although whale faecal samples were collected as soon as the whales defecated, the dilution and potential sinking of faecal material would significantly influence the Fe concentrations measured in these samples. In addition, there are no direct measurements of the daily krill consumption rates by baleen whales. However, tag data suggests that humpback whales in this region spend the vast majority of their time foraging (Friedlaender et al. 2013, Friedlaender et al. 2016a, Tyson et al. 2016) and remain in this state for extended periods of time punctuated by short transits between feeding areas (Friedlaender et al. 2016b). Daily krill consumption estimates for humpback whales range from 694 – 874 kg day^{-1} wet weight and are calculated based on a number of factors (e.g. using daily prey consumption from average body weight of 35 g kg^{-1} body weight, and 2%, 2.5% and 3% of body mass, Lockyer 1981 and Reilly et al. 2004). The variability in consumption rates would thus influence the amount of Fe digested and recycled.

5.5.1.1 Potential processes influencing bioavailability of faecal dFe

Scavenging removes dFe when concentrations exceed the solubility (Johnson et al. 1997, Gordon et al. 1998, Boyd and Ellwood 2010, Saito et al. 2013), which is controlled by chemical speciation according to the environmental conditions, especially the presence of Fe-binding ligands. While the organic complexation of faecal dissolved Fe was not measured in this project, most of the dFe (99%) in the Southern Ocean is complexed by organic ligands (Boye et al. 2001, Croot et al. 2004, Boye et al. 2010, Ibisanmi et al. 2011, Thuróczy et al. 2011). Although the concentration of Fe complexing ligands in Southern Ocean waters is low (0.7 ± 0.2 nmol L^{-1}, Boye et al. 2011), other sources of organic ligands could be important.

Antarctic sea ice contains some of the highest concentrations of organic ligands (4.5 – 72 nmol L^{-1} of sea ice), potentially due to the high ice-associated algal and bacterial production (Lannuzel et al. 2015). Consequently, Southern Ocean seawater may not contain sufficient organic ligands to bind with a large pulse of faecal dFe, however release of recycled Fe close to the ice edge may increase the availability of faecal Fe through binding with organic ligands produced in sea ice. Marine animals could also be a source of organic ligands. Zooplankton have been demonstrated to produce Fe binding ligands (Sato et al. 2007). Accordingly, whales could release organic ligands produced by whale enterobacteria in their faecal material (e.g. enterobactin, a strong siderophore, which is produced by bacteria such as *Escherichia coli* Kraemer 2004, Butler and Martin 2005) resulting in the release of organically complexed Fe in their faecal material, however this has yet to be demonstrated.

In terms of chemical forms, Fe^{2+} is considered to be the more bioavailable fraction, due to the low solubility of the thermodynamically stable Fe^{3+} redox species. In copepods and krill, the acidic digestion process has been demonstrated to aid in the solubilization of Fe (Hutchins and Bruland 1994, Barbeau et al. 1996, Tovar-Sanchez et al. 2007, Schmidt et al. 2016). In vertebrates, HCl and pepsin in the stomach coupled with the low oxygen environment in the intestine would favour the reduction of Fe^{3+} to Fe^{2+} (Slijper 1962, Naikare et al. 2006). Although in an oxygenated environment Fe^{2+} could rapidly be oxidised to Fe^{3+}, if some of the dFe fraction

of whale faeces is released already bound to ligands (similar to that of zooplankton), then the dissolved Fe solubility, bioavailability, and residence time would be greatly increased.

Dissolved Fe can be further partitioned into small soluble species and larger colloidal forms (not measured in this study). Within the dFe phase, soluble Fe may be more bioavailable than the more chemically dynamic colloidal Fe (Wu et al. 2001), and therefore may have a greater control over ocean primary productivity, because the colloidal fraction could aggregate into larger particles, scavenge Fe and settle to the ocean floor (Honeyman and Santschi 1989, Gordon et al. 1998, Wu et al. 2001, Boyd and Ellwood 2010). Conversely, Hassler et al. (2011a) demonstrated the role of organic ligands in enhancing the colloidal fraction within the water column. Dissolved Fe could also be scavenged by other sinking particles, or precipitate from the water column (Wu et al. 2001, Boyd and Ellwood 2010, Fitzsimmons and Boyle 2014). However, complexation with organic ligands can protect dFe from particle scavenging and precipitation (Street and Payton 2005, Fitzsimmons and Boyle 2014) and bacteria can remineralise this sinking fraction and increase dFe concentrations again (Boyd and Ellwood 2010).

Lateral transport of dFe is an important mechanism in supplying Fe to Fe-limited regions. The high dFe concentrations between 1,000 to 2,000 m in the tropical Pacific has been attributed to lateral transport from hydrothermally derived dFe ~500 km away (Boyle et al. 2005, Wu et al. 2011). Lateral supply was also observed in the plume region around the Kerguelen Plateau, whilst vertical supply was the dominant source on the plateau (Bowie et al., 2015). In addition, the lateral supply of dFe from the Kerguelen shelf was higher than the vertical upward dFe flux, and plays a significant role in the phytoplankton bloom observed around Elephant Island (South Shetland Islands; Dulaiova et al., 2009). Assuming that the solubility of the dFe pool in whale faeces is stabilized by organic complexation, whale faecal dFe could also be transported laterally great distances and play an important role in supplying Fe to distal HNLC regions of the Southern Ocean.

5.5.2 Particulate fraction (>0.2 μm)

The particulate fraction constituted the dominant pool of the total Fe in these samples. Generally, Fe concentrations were highest in particles >60 μm (1,815 – 20,784 nmol L^{-1}), followed by particles between 10 and 60 μm (702 – 1,352 nmol L^{-1}) and lastly between 0.2 and 10 μm (389 – 2,507 nmol L^{-1}) (Table 5.3, Figure 5.2). Except for sample 1 where the highest concentrations were observed in the 0.2 – 10 μm fraction, the other samples demonstrated a decline in Fe concentrations with decreasing size fractionation (Table 5.3 and Figure 5.2). The large variation in Fe concentrations between particulate fractions would again reflect the natural variability in the sample, as well as variability in feeding rates and/or assimilation efficiency, and timing of collection as mentioned earlier. Particle aggregation may also increase the Fe concentration in particles >60 μm.

The concentration of total pFe in the faecal materials was comparable to concentrations reported elsewhere for Antarctic land-fast sea ice and marine ice (Herraiz-Borreguerro et al. 2016, van der Merwe et al. 2011b, Lannuzel et al. 2014); lower than in hydrothermal source solutions (James et al. 2014) and krill faecal pellets (Ratnarajah et al. 2016b); but higher than all other Southern Ocean sources including aeolian deposition, surface snow, brine, pack ice, continental ice and sea ice (Table 5.4). Although the pFe in krill faecal pellets is considerably higher than in whale faecal material, the larger size of krill faecal pellets would result in faster sinking rates, decreasing the residence time of Fe in surface waters.

5.5.2.1 Potential processes influencing bioavailability of faecal pFe

The particulate fraction is generally considered less bioavailable for uptake by phytoplankton due to its refractory composition, but could be converted into a more bioavailable pool through recycling of the biogenic pFe in surface waters, leaching of pFe, and remineralisation, or conversely be rapidly removed through aggregation and precipitation. The molar Fe/Al ratio is a useful proxy for estimating the lithogenic and biogenic composition and enrichment of elements in particles (Lannuzel et al. 2011b, Lam et al. 2015, Ohnemus and Lam 2015), which can then be used to help predict the lability of specific elements found in those particles.

Iron and Al are abundant in the Earth's crust and in continental and marine sediments, and although abundances vary, the mean crustal ratio of Fe/Al is approximately 0.2 mol mol^{-1} (Wedepohl 1995, Rudnick and Gao 2003). The Fe/Al molar ratios for the 3 whale faecal material samples measured here were found to be between 0.4 – 0.99 mol mol^{-1}. Assuming that Al is solely lithogenic in origin (however Al can be scavenged leading to an overestimate in lithogenic contribution – see Ohnemus and Lam 2015), and using the Fe/Al molar ratio of 0.2 (Wedepohl 1995), the lithogenic PFe (>0.2 μm) contribution was calculated as (100*[PAl]) * (0.2/[PFe]). These calculations suggest that between 20 – 50% of the faecal material is lithogenic in origin (Table 5.5). The biogenic PFe is considered as the difference between total PFe and lithogenic PFe, and ranged from 50 – 80% (Table 5.5). The enrichment factor (EF) determined as $([PFe]*[PAl])_{faecal\ sample}$ / $([PFe]*[PAl])_{crust}$ was calculated at 2, 5 and 4 for faecal sample 1, 2 and 3 respectively. Elements with EF > 3 – 5 suggest an enrichment of Fe in the water column whereas elements with EF < 3 – 5 is considered to fall within the natural variability inherent to crustal material (Duce et al. 1983). Although the Fe/Al ratios suggest a high biogenic load, the EF factors point towards natural variability in the samples.

The main prey of baleen whales, Antarctic krill, were found to be feeding on the seabed in the Scotia Sea (Schmidt et al. 2011). Therefore, the lithogenic composition in whale faecal material could be attributed to benthic feeding by their prey, Antarctic krill or by accidental feeding of resuspended lithogenic sediments by the whales themselves when they filter krill. The biogenic fraction in whale faecal material from this study could be due to the Fe that had been incorporated into the krill body. The processing of both biogenic and lithogenic material by whales during digestion likely renders all particulate Fe more bioavailable before being defecated at the surface. Furthermore, the particulate Fe in whale faecal material released in surface seawaters could be further broken down and released by both micro- and meso-zooplankton (Sarthou et al. 2008, Strzepek et al. 2005, Maranger et al. 1998, Barbeau et al. 1996, Hutchins and Bruland 1994), heterotrophic bacteria (Strzepek et al. 2005, Sarthou et al. 2008, Boyd et al. 2010) and viruses (Mioni et al. 2005), thus making it even more bioavailable to Fe-limited plankton.

The dissolution of digested and defecated lithogenic pFe could be an important source of dFe in this region (Blain et al. 2007, van der Merwe et al. 2014). For example, the high vertical dFe supply in the naturally fertilised region of the Kerguelen Plateau did not meet the dFe demand of phytoplankton, and the missing Fe supply could have been met through the extended dissolution of leachable Fe from resuspended marine particles (Blain et al. (2007). Similarly, the dissolution of pFe from faecal material, although largely biogenic in origin, could play an important role in the supply of dissolvable Fe into surface water prior to sinking.

To test the instantaneous and prolonged solubilization of Fe in faecal material, the three size fractions were subjected to seawater leach experiments over a 12-hour period from a single sample ("Sample 3"). The leaching experiment demonstrated that most of the labile Fe is released within the first 5 minutes (Figure 5.3). At the 0.2 μm size fraction, approximately 5% of the material is leached into the dissolved pool. Approximately 7% is leached within the first 5 minutes from the 10 μm filter, and only approximately 1% from the 60 μm filter. The solubility of faecal particles decreased over time with approximately 1% being leached after 12 hours for the 0.2 μm size fraction, 2.5% for the 10 μm size fraction and ~0 % for the >60 μm size fraction. Comparatively, the solubility of atmospheric dust particles is between 0.5 – 80% (Mahowald et al. 2005, Winton et al. 2015). However, these leaching experiments were performed using different leach solutions (UHP and weak acid such as acetic acid and hydroxylamine hydrochloride), and at different temperatures (heating step of 90 – 95°C, Winton et al. 2015), which would influence solubility estimates (Wu et al. 2007, Schroth et al. 2009, Aguilar-Islas et al. 2010, Mendez et al. 2010, Gao et al. 2013, Morton et al. 2013, Winton et al. 2015). A similar seawater leach experiment using particles in sea ice (> 0.2 μm) at 4°C found a maximum of 6% solubility (Kanna et al. 2016).

Although the % solubility of faecal particles is at the lower end of the scale, there is still a high concentration of dFe being leached from these particles. For instance, in the first 5 minutes, 51 nmol L^{-1} of Fe is leached from the 0.2 μm filter, whilst 103 nmol L^{-1} and 143 nmol L^{-1} is leached

from the 10 μm and 60 μm fractions respectively. The concentration of dissolvable Fe here could be an underestimate due to adsorption of dFe to container walls (Wu et al. 2007). In laboratory experiments, the ratio between particle load and leach solution have been shown to contribute to the variability in the estimates of aerosol Fe dissolution in seawater, where higher particle load resulted in a decrease in percentage Fe released (Bonnet and Guieu 2004). However, this is not likely to be a problem in the open ocean, where the faecal material is dispersed over a large volume during defecation.

It is important to compare the leaching rates of pFe against the sinking rates to gain a better estimate of the residence time in the water column. The sinking velocity of pFe is a function of its particle density and size. The density of organic matter (1,060 kg m^{-3}, Logan and Hunt 1987) is close to that of water (1,027 kg m^{-3}). Using Stoke's Law for the sinking of spherical particles, the sinking rate of faecal particles in seawater was calculated for a particle with a diameter of 0.2 μm, 10 μm and 60 μm (using gravitational acceleration of 9.81 m s^{-2} and dynamic viscosity of seawater of 1.88 x 10^{-3} kg s^{-1} m^{-2}). A particle of 60 μm would sink at a rate of 3 m day^{-1}, while a particle of 10 μm would sink at a rate approximately 0.08 m day^{-1}. At the smallest fraction, a particle of 0.2 μm would sink at a rate of 3.3 x 10^{-5} m day^{-1}.

Assuming a euphotic depth of 100 m, the larger particles (60 μm) would remain in the euphotic zone for 33 days, whilst the medium-sized particles (10 μm) would remain for 3.4 years (1,250 days), and the smallest particles (0.2 μm) would remain for >10 years. However, these sinking rate estimates are only approximations and assume that particles are spherical. Furthermore, particle aggregation will reduce the residence time in surface waters, the exact particle density for these samples is unknown, and rapid dilution in seawater, dissolution, scavenging, uptake and remineralisation processes will heavily influence the magnitude and settling rate of sinking particles.

As the pFe fraction sinks due to a combination of scavenging and gravitational forces, the bacterial community gradually remineralizes the organic matter, consequently returning Fe to the

dissolved phase (Boyd and Ellwood 2010). Furthermore, using particles >53 μm, Boyd et al. (2010) demonstrated that bacteria are capable of releasing weak Fe-binding ligands during particle remineralisation. Therefore as faecal pFe sinks, not only will zooplankton and bacteria rapidly recycle the biogenic pool, the fraction that sinks will also be remineralised by the bacterial community.

Lateral transport of pFe has been demonstrated to play a crucial role in the supply of Fe to Fe-limited waters (Johnson et al. 2005, Lam et al. 2006, Lam and Bishop 2008, Bowie et al. 2009). The wintertime phytoplankton bloom observed in the HNLC region of the subarctic North Pacific Ocean has been attributed to the lateral transport of pFe from the continental margin of the Aleutian Islands (Lam et al. 2006). Similarly, the high subsurface concentrations of pFe southeast of Tasmania is thought to be from shelf sediments laterally transported from Tasmania or from eastern mainland Australia, or Australian dust initially deposited in waters north of the sampling region and transported laterally towards the southeast (Bowie et al. 2009). Consequently, faecal-derived pFe could also be transported laterally to fertilise Fe-limited HNLC waters further afield.

The dilution of faecal samples in seawater, as well as particle aggregation and precipitation will also influence the bioavailability of Fe to phytoplankton. The rate of defecation or exact volume of faecal material defecated by a whale is unknown, however the image of a defecating humpback whale (Figure 5.1a) suggests that it is voluminous. The pFe sinking rates calculated above are for individual particles. However, a major removal pathway of pFe from surface waters is through aggregation into larger particles (Frew et al. 2006, van der Merwe et al. 2014). van der Merwe et al. (2014) demonstrated that the aggregation of particles onto phyto-aggregates resulted in a 70% decrease in the pFe concentration within the mixed layer. In addition, when Fe concentrations exceed the solubility, Fe will be removed through scavenging and precipitation, as discussed earlier. Therefore, much of this faecal pFe could be rapidly exported via sinking aggregates, and a true concentration of bioavailable Fe will reflect a balance between the accumulation and removal mechanisms discussed here.

5.6 Conclusion

The distribution of bioavailable Fe in the ocean reflects a balance between Fe sources, biological uptake, speciation (chemical and physical), scavenging, precipitation, aggregation, remineralisation and lateral transport. Therefore it is critical to understand the role of marine animals in Fe recycling to better constrain biogeochemical budgets of Fe in the Southern Ocean. This study provided the first quantitative estimate on the physical speciation of Fe in recycled organic material. Biological recycling of Fe through large marine animals such as whales could play an important role in the resupply of Fe to the Fe-limited waters of the Southern Ocean, and historical commercial whaling practices could have significantly impacted this recycling efficiency.

The measured concentrations of dFe and pFe in whale faecal material are some of the highest compared to all other sources into the region, and a time series leaching experiment suggests that Fe is continually leached from these particles over time. Although the particulate fraction dominated the total Fe pool, zooplankton and microbial solubilisation of biogenic pFe, coupled with leaching of pFe over time and the slow sinking rates of organic matter, may imply that the faecal material would be rapidly recycled in the surface for biological uptake. Furthermore, as demonstrated in other studies, lateral transport of dFe and pFe could be an important mechanism in transporting Fe to other Fe-limited regions further away. There is a constant transfer dFe to pFe and vice versa. It is now important to: (i) determine if whales, like zooplankton, release organic Fe-binding ligands in their faecal material that would keep the dFe in suspension in the water column; (ii) understand the uptake response of phytoplankton to a faecal-derived Fe source; (iii) consider the supply of Fe by whales compared to other sources in the region, and (iv) examine the influence of historical whaling practices.

5.7 Acknowledgements

We would like to thank Pier van der Merwe, Shaun Brooks, Tom Holmes, Prof. Tom Trull and the two anonymous reviewers for their comments and suggestions during the development of this project and manuscript. Lastly, to Tino Bowie-Proemse for his support. This study was supported by the Australian Government's Cooperative Research Centres Programme through the Antarctic Climate and Ecosystem Cooperative Research Centre, the Institute for Marine and Antarctic Studies, and the Holsworth Wildlife Research Endowment. Access to SF-ICP-MS instrumentation was supported through ARC LIEF funds (LE0989539).

6 General Discussion

6.1 Global summary

This dissertation research builds on ideas proposed in Smetacek and Nicol (2005) and Nicol et al. (2010), and has added significantly to our understanding of the influence of Antarctic krill and baleen whales in recycling Fe, and other biologically important trace elements in the Southern Ocean. During this research project, 3 peer-reviewed articles have been published (Chapter 2 – 4), with another currently under review (Chapter 5).

In Chapter 2, the concentration of Fe and other biologically important trace elements such as zinc (Zn), cadmium (Cd), cobalt (Co), manganese (Mn), copper (Cu) and phosphorus (P) were measured in specimens of whole Antarctic krill, and whale muscle and faecal material. The aim of this chapter was to examine if Antarctic krill and baleen whales do play a role in the cycling of trace elements in the Southern Ocean through their diet and subsequent defecation. Although Fe is the most limiting element in these large HNLC areas of the Southern Ocean, other elements are also required by phytoplankton for a suit of functions such as Fe and Mn for carbon (C) fixation; Mn is required by the water oxidizing complex of photosystem II; Zn, Cd and Co for CO_2 acquisition; Zn and Cd for silica uptake; Co and Zn as calcifiers; Cu and Fe for nitrification, denitrification, and organic N utilization; and Cu for methane oxidation (Morel et al. 2003, Morel and Price 2003).

Here it was demonstrated that Antarctic krill are rich in all these elements, however the relative concentration of each of these elements within the krill varies, primarily due to differential uptake and utilisation by the krill. For instance, crustaceans require Cu for their respiratory pigment, hemocyanin (Spicer and Saborowski 2010), however Cu is little concentrated by phytoplankton (Annett et al. 2008). Consequently, Antarctic krill must consume vast amounts of phytoplankton to meet their demand for Cu. On the other hand, the concentration of Fe in Antarctic krill is significantly higher compared to Southern Ocean surface seawater, and the Fe:C

ratio in krill is 3 times higher than phytoplankton growing in low Fe conditions (Twining and Baines 2004) suggesting that they are storing Fe.

The Fe, and other biologically important elements stored in krill are transferred into the whales as they are consumed. Baleen whales spend between 4 – 6 months a year feeding in the Southern Ocean. Some of these elements may be preferentially retained in the muscle for use. For instance, Fe is required for muscle growth (Ordway and Garry 2004), and may be retained by young growing whales, pregnant or injured whales. However, as adult whales mainly build blubber (fat) to last them through the mating season in warmer waters where food is almost non-existent, much of the Fe gets defecated. Iron concentrations in whale faeces were over 10 million times higher compared to background seawater levels. Conveniently, baleen whales defecate a liquid slurry that rapidly spreads in the ocean surface. It is predicted that this faecal material could be an important source of recycled Fe that could stimulate phytoplankton growth.

A preliminary sensitivity analysis model was built in Chapter 3 to explore if baleen whales and Antarctic krill could stimulate the growth of phytoplankton in the Southern Ocean. A local sensitivity analysis was applied to the model, which allowed for a range of parameters (minimum, mean and maximum) from the literature to be used. Where there were no estimates for certain parameters, conservative assumptions were made based on current understanding of Fe chemistry. The objective of this model was two-fold: to determine 1) if baleen whales and Antarctic krill could influence phytoplankton growth in the Southern Ocean, and 2) the influence of parameter uncertainty on the estimated contribution.

The preliminary model suggested that Fe-rich defecation by baleen whales and Antarctic krill could influence the growth of phytoplankton in the Southern Ocean, but only when all the parameter estimates were at their upper limits. The estimated contribution was heavily influenced by five parameters: (1) Fe content in Antarctic krill, (2) krill consumption rates by whales, (3) persistence of whale faecal Fe in the photic zone, (4) the bioavailability of this retained Fe, and (5) the C:Fe ratio of phytoplankton.

The most influential parameter as determined by the preliminary model, "Fe content in Antarctic krill" was examined in Chapter 4. Antarctic krill specimens were collected over 3 research voyages to Prydz Bay, Antarctica, and dissected to examine the cause of the large range of Fe concentrations in Antarctic krill reported in the literature (6 – 190 mg kg^{-1}). The concentration of Fe was measured in krill stomach, digestive gland, muscle, exoskeleton, body (excluding stomach and digestive gland only) and faecal material. This study clearly demonstrated that much of the Fe is being stored in the stomach (6 – 98 mg kg^{-1}) and digestive gland (14 – 82 mg kg^{-1}) compared to the muscle (4 – 7 mg kg^{-1}), exoskeleton (6 – 15 mg kg^{-1}) and body (4 – 18 mg kg^{-1}). The Fe stored in the digestive organs is being excreted as faecal pellets (683 – 1,039 mg kg^{-1}).

This suggests that Antarctic krill consume great quantities of Fe but require little Fe for their metabolic processes; consequently the large variation reported in the literature could be due to their feeding patterns (i.e. seasonal and regional differences in the quality and quantity of their diet or methodological and analytical differences). Furthermore, calculations comparing the results obtained here with those measured in Schmidt et al. (2011), further suggest that despite the relatively small size of the stomach and digestive gland, the high Fe concentrations within these organs can influence the overall Fe concentration measured in whole krill specimens.

Finally, the third and fourth most influential parameters in the preliminary model, "persistence of whale faecal Fe in the photic zone" and "the bioavailability of this retained Fe" was investigated in Chapter 5. Whale faecal samples were opportunistically collected during the summer feeding season around Palmer Station, Anvers Island. The bioavailability of Fe reflects the ability of phytoplankton cells to acquire Fe, and the size fractionation between the dissolved (<0.2 μm) and particulate (>0.2 μm) fraction is commonly used to determine element bioavailability. Here, the size fractionations of Fe from 3 whale faecal samples were examined at 4 different size fractions (<0.2μm, 0.2 – 10 μm, 10 – 60 μm and >60 μm).

It was consistently observed that the particulate fraction (>0.2 μm = 5,026 – 22,526 nmol L^{-1}) dominated the total Fe pool, however the concentration of dFe (186 – 754 nmol L^{-1}) in whale faecal material was much higher compared to background seawater concentrations (0.1 – 0.5 nmol L^{-1}, Tagliabue et al. 2012), and many other sources in the region, but is comparable to marine and continental ice (Herraiz-Borreguero et al. 2016b). Furthermore, the concentration of dFe in whale faecal material is greater than the solubility of Fe in Southern Ocean surface seawater. The solubility of Fe is a function of the organic ligands present in the seawater. The concentration of Fe binding ligands in the Southern Ocean waters is low (0.7 ± 0.2 nmol L^{-1}, Boye et al. 2011), but the concentrations of organic ligands are higher in Antarctic sea ice (4.5 – 72 nmol L^{-1}, Lannuzel et al. 2015). Therefore, the seawater may not contain enough organic ligands to bind with this large pulse of faecal dFe, but the release of faecal material closer to the ice edge may increase the bioavailability of faecal Fe. In addition, it is unclear if whales release organic ligands in their faecal material, and if so, the type and concentration of ligands, which would influence the residence time of dFe in surface waters.

Although most of the faecal material consisted of particles >0.2 μm, sinking rates calculated for these particles suggested that they would remain in the euphotic zone (assuming euphotic depth of 100 m), for 33 days for particles with a diameter of 60 μm, 3.4 years for particles with a diameter of 10 μm, and >10 years for a particle with diameter of 0.2 μm. These estimates are only approximations because the exact density is unknown, and it is assumed that these particles are spherical. However, this suggests that a large fraction of faecal Fe may remain in the euphotic zone. Furthermore, Fe could be leached from these particles over time. The leaching experiment demonstrated that most of the leaching occurred within the first hour. While Fe concentrations in whale faecal material is extremely high, the bioavailable fraction is further constrained by additional processes such as scavenging, precipitation, aggregation, remineralisation, chemical speciation, and lateral transport.

6.2 Review of current literature and future work

This dissertation has demonstrated that Antarctic krill and baleen whales play an important role in the cycling of Fe, and other biologically important trace elements in the Southern Ocean. Within this rapidly evolving field, many concurrent field and modelling investigations were performed, summarised here.

6.2.1 Antarctic krill

The first study investigating the role of Antarctic krill in the biogeochemical cycling of nutrients in the Southern Ocean was presented in Tovar-Sanchez et al. (2007). Using shipboard incubation experiments, the authors demonstrate that the presence of Antarctic krill increased the Fe concentrations by 0.2 – 4.3 nmol L^{-1} d^{-1}. The high Fe concentrations measured in krill faecal pellets in Chapter 4 would corroborate this finding. In addition to their role in recycling Fe, this dissertation research suggests that prior to Fe processing in krill digestive organs and excretion via faecal pellets, Antarctic krill also act as a reservoir of Fe to be consumed by higher tropic levels.

In Schmidt et al. (2011), the authors demonstrated that benthic feeding by Antarctic krill could have significant implications on the vertical transfer of Fe. The stomach of Antarctic krill sourced from the Scotia Sea contained high concentrations of refractory Fe. Similarly, in Schmidt et al. (2016), the authors demonstrated that Antarctic krill sourced from South Georgia ingest large quantities of lithogenic Fe originating from glacial outlets, and excrete >90% of this in their faecal pellets. Conversely, in Antarctic krill sourced from Prydz Bay for this dissertation research, the Fe concentration in krill stomach was comparatively lower, suggesting surface feeding instead of benthic feeding. Nevertheless all these studies clearly suggest that Antarctic krill play an important role in both the storage, and recycling of Fe in the Southern Ocean.

Schmidt et al. (2016) suggests that in years of high krill abundance, greater phytoplankton blooms downstream of South Georgia are observed. However, it remains unclear if krill boosts

phytoplankton growth in a particular region through the provision of bioavailable Fe, or indeed are driven to that region because of the high primary productivity (Ratnarajah and Bowie 2016).

The high Fe concentrations measured in Antarctic krill faecal pellets (Ratnarajah et al. 2016b, Schmidt et al. 2016), and the leaching of Fe from these faecal pellets (Tovar-Sanchez et al. 2007, Schmidt et al. 2011) suggest that Antarctic krill could be an important source of recycled Fe into the Southern Ocean that could stimulate primary productivity. However, the bioavailability of krill faecal Fe, and its eventual uptake by phytoplankton is unknown (Ratnarajah and Bowie 2016). The bioavailability, and uptake is a function of the sinking rate of faecal pellets. Sinking rate of Antarctic krill faecal pellet from the literature is between 27 to 1218 m day^{-1}, with the variability mainly driven by pellet diameter and density. Future studies should investigate the bioavailability of krill faecal material coupled with its sinking rate to gain a better understanding on the role of Antarctic krill in the biogeochemical cycling of Fe in the Southern Ocean (Ratnarajah and Bowie 2016).

6.2.2 Baleen whales

Only one other study investigated the Fe concentration in whale faeces, and the results from that study formed the backbone to this dissertation research. In Nicol et al. (2010), the authors demonstrate that baleen whales, and sperm whales are an important component in the cycling of Fe in the Southern Ocean, and could be a significant contributor to Fe in Southern Ocean surface seawater. As summarised in Section 6.1, it is now clear that baleen whales are a source of recycled Fe into Southern Ocean surface seawater, and the concentration of dFe in whale faeces is beyond the solubility of Fe in surface seawater. To fully understand the role of marine animals in supplying bioavailable Fe for uptake by phytoplankton, future research should investigate if baleen whales release organic ligands in their faecal material, therefore keeping much of the Fe in suspension for uptake by phytoplankton.

Smith et al. (2013) demonstrated that the Fe-rich faecal material from pygmy blue whales stimulates the growth of three phytoplankton species – *Dunaliella tertiolecta*, *Chaetoceros pendulus* and *Phaeocystic Antarctica* in laboratory cultures. Ultimately, the most pressing question is to determine if this translates to natural phytoplankton assemblages in the Southern Ocean through *in situ* incubation experiments.

6.2.3 Other marine animals

In addition to Antarctic krill and baleen whales, studies have also suggested that other marine animals would play an important role in the recycling of Fe to Fe-limited regions in the Southern Ocean. In Wing et al. (2014), the authors demonstrate that seabird guano, and the faecal material of the Hooker's sea lions also contribute to the Fe enrichment around Auckland Islands (New Zealand). Furthermore, incubation experiments from seawater collected from the Sub-Tropical Frontal Zone, the Sub-Antarctic Zone and Antarctica suggest that seabird guano stimulates primary productivity in this region (Shatova et al. 2016). It is important to now explore the role of other marine animals in the region to develop a greater understanding on the ecological role of marine animals, and the biogeochemical cycling of Fe in the Southern Ocean and Antarctic region.

6.2.4 Modelling

A number of models have been developed to investigate the influence of baleen whales on primary productivity, and carbon sequestration in the Southern Ocean. Lavery et al. (2010) proposed that the Fe-rich faecal plume of sperm whales could sequester 4×10^5 tonnes of C per year to the ocean floor. It is difficult, if not impossible; to predict the potential carbon sequestration through faecal Fe induced primary productivity. Multiple artificial Fe fertilisation experiments have demonstrated that the addition of Fe would stimulate the growth of phytoplankton (see synthesis in Boyd et al. 2007). However, there is no conclusive evidence on carbon sequestration from these studies. For instance, during IRONEXII (Coale et al. 1996, Bidigare et al. 1999), there was a seven-fold increase in export production, however this was not observed during the SOIREE experiment, even though an increase in primary productivity was

observed (Boyd et al. 2000). This is due to an interplay between processes that control the persistence (e.g. binding with organic ligands, remineralisation etc.), and export (scavenging, precipitation, aggregation, gravitational sinking, consumption by grazers etc.) of Fe and phytoplankton cells from the water column (Boyd and Ellwood 2010).

Subsequently, Lavery et al. (2014) demonstrated that the biological recycling of Fe by blue whales would stimulate primary productivity leading to increased krill stocks, contrary to the traditional thought that whales compete with fisheries. In a more complicated Fe recycling model developed by Maldonado et al. (2016), which incorporates the entire Southern Ocean food web, the authors corroborate the findings from Lavery et al. (2014), suggesting that the biomass of krill, salps, benthos, bacterioplankton and microzooplankton would have had to be higher in the pre-whaling era compared to the present. Along the same lines, the model presented in Chapter 3 suggested that the biological recycling of Fe by Antarctic krill and baleen whales was self-sustaining. The preliminary model did not investigate the 'krill surplus' hypothesis (i.e. the removal of whales during in the early to mid 1900's resulted would result in a surplus of Antarctic krill), but it was proposed that the recycling of Fe could stimulate sufficient primary productivity, beyond the metabolic requirements of krill.

The Ecopath model proposed in Maldonado et al. (2016) suggested that microzooplankton had the largest contribution to Fe recycling in the Southern Ocean. It was suggested that the contribution of whales to the recycling of Fe in the Southern Ocean was insignificant compared to planktonic consumers, however the removal of whales may have impacted the structure and productivity of the Southern Ocean marine ecosystem in ways yet unknown. Similarly, the model presented in Chapter 3, which is a diet dependent model only exploring the relationship between Antarctic krill and baleen whales, demonstrates that baleen whales may not be a significant contributor, when all parameters were at the lower limit, but it could have an effect when all parameters were at their upper limits.

Furthermore, the ECOPATH model fails to explore the mode of supply of Fe (i.e. liquid slurry at the surface by baleen whales compared to faecal pellets by zooplankton and krill) but states that the faecal material released from grazing microzooplankton would be considered more bioavailable compared to faecal pellets released by carnivorous zooplankton and krill, which has yet to be empirically demonstrated. The authors acknowledge that future studies should investigate the residence time and bioavailability of faecal Fe from various sources. Based on laboratory analysis presented in Chapter 5, it can be concluded that whales are an important recycler of Fe in the Southern Ocean as the concentrations of dFe in whale faecal material is extremely high, comparable only to marine and continental ice. Consequently, future models should aim to include mode of supply, as it could drastically alter model conclusions.

This research has demonstrated that baleen whales and Antarctic krill are key components in the cycling of Fe in the Southern Ocean. It is now important to determine 1) if Antarctic krill increase phytoplankton growth in a particular region through the provision of bioavailable Fe, or are driven to that region because of the high primary productivity, 2) the bioavailability of krill faecal Fe, and its eventual uptake by phytoplankton, 3) if baleen whales release organic ligands in their faecal material, 4) the response of natural assemblages of phytoplankton to whale faecal material, 5) the influence of other marine animals on the Southern Ocean iron cycle and 6) to further constrain models as empirical evidence becomes available to gain an understanding on the biogeochemical role of marine animals in the cycling of Fe in the Southern Ocean.

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